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The effect of treatment regimens on salivary cortisol levels in patients with chronic musculoskeletal disorders

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ABSTRACT

Background: Increased levels of circulating cortisol have been associated with pain severity in patients with chronic musculoskeletal disorders (CMD). Little is known about the potential association between pain management and salivary cortisol alterations in CPM patients treated with different regimens.
Objectives: This prospective feasibility study aimed to determine the effect of two treatment regimens in comparison with sham therapy on pain intensity and disability and salivary cortisol concentration (SCC) in patients with CMD.
Methods: Thirty patients were randomly assigned to 3 groups of 10: two experimental groups (A and B) and a control group (C). The experimental groups followed physiotherapy treatment (A) or acupuncture (B), while the control group (C) followed a sham therapy for 10 sessions. Pain data were collected using the Chronic Pain Grade (CPG) questionnaire and SCC was measured by enzyme-linked immunosorbent assay at pre- and posttreatment.
Results: Repeated-measures analysis of variance showed that patients treated with acupuncture experienced greater decreases in pain intensity/pain disability ($P < 0.05$) than the physiotherapy and sham therapy groups. No statistical differences were found between the three groups for the SCC outcome variable. Bonferroni adjustments showed that the mean values of SCC were significantly decreased at posttreatment ($P < 0.05$) across the three groups.
Conclusion: There was a significant decrease in both pain and cortisol outcomes at posttreatment in patients with CMD. Because of the limitations of this study, we cannot draw conclusions regarding whether the lower SCC could be an indication of pain reduction in patients with CMD.

1. Introduction

Cortisol is an essential steroid hormone produced in the adrenal cortex within the adrenal gland (Goodin et al., 2012; Raff, 2011; Chan and Debono, 2010). In humans, the levels of cortisol in the blood and saliva have diurnal variation, peaking in the early morning (approximately 8 a.m.) and reaching their lowest level between midnight and 4 a.m., or 3–5 h after the onset of sleep (Raff, 2011). Cortisol concentration is also related to stress and low blood glucose concentration. The hypothalamic-pituitary-adrenal (HPA) axis has been proposed as a potential mediator of cortisol production. Activation of the HPA axis has been associated with increased pain severity in patients with chronic musculoskeletal pain conditions (Goodin et al., 2012; McBeth et al., 2005; Neeck and Riedel, 1999; Neeck, 2000). However, previous clinical studies have found an inverse relationship between cortisol and chronic musculoskeletal pain. A higher cortisol concentration was associated with less severe pain (Carnes et al., 2007; Al'Absi et al., 2002), and a lower cortisol concentration was associated with higher pain levels (Goodin et al., 2012; Geiss et al., 1999). Thus, it appears that additional research is needed to further elucidate the nature of the relationship between cortisol concentration and chronic musculoskeletal pain.

Chronic musculoskeletal pain is typically caused by chronic musculoskeletal disorders (CMDs). CMD has been defined as a condition in which pain lasts longer than 3 months (Phillips and Clauw, 2013), and its prevalence has been estimated to be ~10% of

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the population (Schell et al., 2008; Verhaak et al., 1998). Pain symptoms in CMD are among the top five reasons why patients visit clinics and emergency departments (Penney et al., 2016). CMDs can be present in multiple locations within the musculoskeletal system and have been associated with a great negative impact on patients' functioning (Kalameri et al., 2009, 2008) and disability (Generaal et al., 2014; Carnes et al., 2007; Davies et al., 1998). Evidence-based research in CMD has proposed that multifaceted therapy regimens (physiotherapy, acupuncture, exercise, patient education, cognitive therapy, and medications) may help to decrease pain levels (Penney et al., 2016; Pereira et al., 2016; Mason et al., 2004). Despite many systematic reviews, there is no structured methodological quality appraisal of specific physiotherapy regimens in managing musculoskeletal pain conditions. Many therapeutic approaches have been suggested based on painful areas, diagnostic accuracy, treatment effectiveness, use of healthcare resources, and patient satisfaction (Demueles et al., 2012; Hush et al., 2011; Hall et al., 2010).

Nevertheless, research on the determination of cortisol concentration in relation to CMD pain is limited, and the findings are inconsistent. In particular, most of the related studies have reported changes in cortisol concentration in relation to stress, somatic pain conditions (Janssens et al., 2012), and cortisol medication (Carnes et al., 2007), and no study has assessed salivary cortisol concentration changes after a specific treatment regimen in patients with painful CMDs.

Thus, the aim of this study was to determine the effect of physiotherapy and acupuncture in comparison with sham therapy on a) pain intensity and disability and b) saliva cortisol concentration in patients with CMD.

Our first hypothesis was that pain levels would be decreased in patients who were treated with different therapeutic regimens in comparison with the control group. Our second hypothesis was that the cortisol concentration would be altered in relation to the effectiveness of pain management by the treatment regimens.

2. Methods

2.1. Participants

This prospective feasibility study was conducted on volunteers from the general population of a public hospital. Thirty patients, 6 men and 24 women, participated in the study (age: 53.96 ± 9.65 years, body mass 67.63 ± 7.42 kg, height 1.66 ± 7.6 cm, and body mass index (BMI) 24.29 ± 3.95 kg/m^2]. All participants had different occupations. The patients were divided into three groups of ten patients each, two experimental (A, B) and one control (C) group, using stratified random choices (by coin flipping) (Table 1).

2.1.1. Inclusion criteria

To ensure group homogeneity, all patients were required to fulfill the following criteria: a) age range between 40 and 65 years, b) presence of CMD on several locations (i.e., the spine, neck, thorax, chest, back, abdomen, pelvis, upper and lower extremities, joints of the arms, hands, legs or feet, mouth, and face) with pain duration >3 months, c) active in their low-level daily activities despite experiencing chronic pain, and d) did not meet any of the exclusion criteria.

Table 1
Demographic characteristics of the three groups (mean ± SD).

	Age (years)	Body mass (kg)	Height (cm)	BMI (kg/m^2)
Group A (n = 10)	55.70 ± 9.86	61.60 ± 7.72	1.63 ± 6.47	22.92 ± 2.79
Group B (n = 10)	54.40 ± 0.08	73.60 ± 6.17	1.70 ± 0.08	25.14 ± 5.07
Group C (n = 10)	53.80 ± 10.08	67.70 ± 7.09	1.65 ± 6.99	24.82 ± 3.69

Group A = physiotherapy group, Group B = acupuncture group, Group C = control group.

2.1.2. Exclusion criteria

The following exclusion criteria were applied: pregnant or breastfeeding women, cancer, high-severity disability level, diagnosed to have sleep disorder or taking sleep medication, circulatory disorders, history of cardiac events, history of metabolic disease or neuropathy, and taking prescribed medications (analgesics, tranquilizers, antidepressants, or other centrally acting agents) that influence the neuroendocrine or immune system or the HPA axis function.

Thirty-nine patients were initially assessed and nine excluded according to their physician's decision based on recent surgery, diagnosis of depression or anxiety disorders, and use of corticosteroid medication. All patients were assessed by the same physician. Written informed consent, which was approved by the Ethics Committee of the University, was obtained from all volunteers who participated in this study. The research protocol was approved by the Ethics Committee of the University (Rec. No. 1617006490, 25/10/16) and by the anesthesiology department of "Sotiria" Athens Chest Diseases Hospital (Rec. No. 6313, 24/3/17). All experimental procedures conformed with the Declaration of Helsinki.

2.2. Main outcome measures

2.2.1. Chronic Pain Grade

Chronic musculoskeletal pain was measured using the Chronic Pain Grade (CPG) scale as a reliable and valid indicator of severity of chronic pain (Von Korff et al., 1992) in patients with CMD. The CPG is an interview-administered questionnaire composed of 7 questions. The CPG inquires about the presence of pain on several locations (i.e., the spine, neck, thorax, chest, back, abdomen, pelvic, upper and lower extremities, joints of the arms, hands, legs or feet, mouth, and face) during the period of 3–6 months prior to the beginning of the study. The 7 questions in the CPG refer to the most painful location and have five grades ranging from grade 0 (*pain free*) to grade 5 (*high disability*), including the following: a) characteristic pain intensity (0–100 score), b) disability score (0–100 score), and c) the indicated points for disability days. All patients in the three groups completed the CPG in two phases: before the beginning (pretest) of the treatment protocols and 6 weeks after the end of the treatment effects (posttest). The CPG scale was completed by an experienced researcher, and the time to complete the full test did not exceed 10 min. The total score of each patient before and after treatment protocols was used for statistical analysis. All patients also completed a self-report based on displayed body charts according to their musculoskeletal pain locations. Each subject reported multiple locations of pain (from 1 with a maximum of 3 pain locations).

2.2.2. Salivary cortisol measurements

The biologically active component of the HPA axis is free plasma cortisol, which is in equilibrium with salivary cortisol (Salimetrics, 2013). Saliva obtained from a commercially available cotton sampling device such as the Salivate, which is easy to use and transport, appears to provide salivary cortisol results that are very reliable predictors of total and free plasma cortisol levels (Salimetrics, 2013). It is recommended to collect the unstimulated mixed saliva samples from 8:00 to 10:00 a.m. because peak cortisol levels are reached at 8:30 a.m. (Chan and Debono, 2010). Hence, each subject's salivary cortisol collection was performed between 8:00 and 10:00 a.m. for all measurements, before the beginning (pretest) of the treatment protocols and 6 weeks after the end of the treatment effects (posttest).

The salivary cortisol sample collection was performed using

salivate swabs 15 min prior to the application of treatment protocols for both the pretest and the posttest (6 weeks later) measurements. More specifically, before starting saliva collection from each participant, the following recommendations were given: 1) Immediately before sample collection, avoid foods with high sugar or acidity or high caffeine content, because they may compromise the assay by lowering saliva pH and increasing bacterial growth (Salimetrics, 2013). 2) Avoid consumption of alcohol, caffeine, nicotine, and medications 12 h before the test (Salimetrics, 2013). 3) Avoid vigorous physical activity and developing any oral diseases or injury. 4) Do not eat a major meal within 60 min of sample collection. 5) Rinse mouth with water to remove food residue and wait at least 10 min after rinsing to avoid sample dilution before collecting saliva. Good saliva collection requires documenting items that may affect results as well as following procedures that avoid the possibility of contaminating saliva with substances that could interfere with the immunoassay. Specifically, the swab was placed in the mouth for 1 min and then transferred into plastic tubes and centrifuged; the collected saliva samples were then analyzed.

Salivary levels of cortisol were determined by standard sandwich enzyme-linked immunosorbent assay using a commercially available kit (Abnova Cor., Taipei City, Taiwan) following the instructions of the user manual. Optical density measurements were performed with a microplate reader (Versamax, Molecular Devices, Sunnyvale, CA, USA) at 450 nm, and calculations were performed using a Soft Max Pro software (Molecular Devices, Sunnyvale, CA, USA). According to the manufacturers, the analytical sensitivity of the assay in terms of the minimal detection limit was 0.0245 ng/mL^{-1}. The intra- and interassay coefficients of variation were 5.8% and 6.4%, respectively. A pilot assay procedure was performed to calculate the concentration of specimen dilution (~1:10) as suggested in order that each run be included within the standard curve (Fig. 1). Using the mean absorbance value for each sample, the corresponding cortisol concentration was automatically determined based on the standard curve (Fig. 1).

2.2.3. Treatment protocols

Subjects were randomly divided into 3 groups (2 experimental and 1 control). Each group was treated with different treatment protocols. All treatment protocols lasted 45 min for each session, and each patient had 10 therapeutic sessions, twice a week, for a total of 6 weeks. The population of this research consisted of general public hospital lists, and public insurance covered the cost of the treatment sessions. Thus, the subjects' level of compliance was 100% in each group. Subjects who did not show up for their treatment session were rescheduled for the next week. The first experimental group and the control group were treated by the same senior physiotherapist experienced in treating musculoskeletal disorders. The second experimental group was treated by the same senior doctor acupuncturist (>15 years postqualification experience). Within the time allocated for this current study, all patients followed rehabilitation programs according to their primary musculoskeletal pain condition. The first experimental group (group A) followed a physiotherapy program based on the decision of the physical therapist in accordance with the physician's diagnosis. The physiotherapy program consisted of 10 min of therapeutic ultrasound (acoustic waves at 1 or 3 MHz and at amplitude densities between 0.1 and 3 W/cm^2), myofascial release (MFR) therapy (Ajimsha et al., 2015; McKenney et al., 2013), and stretching and strengthening exercises (see appendix). The second experimental group (group B) received acupuncture treatment of traditional Chinese medicine (Fu et al., 2014; Vazouez-Mejuto et al., 2014; Cheshire et al., 2013; Kim et al., 2013; DeBar et al., 2011; Hutchinson et al., 2012; Cherkin et al., 2009) (see appendix). The third group, the control (group C), received sham treatment (Benedetti et al., 2011) with disconnected ultrasound and MFR therapy based on simple touch without applying any technical method.

Subjects were randomly assigned to receive the intervention or control treatment, and outcomes were evaluated after the intervention period. Subjects were unaware of the group to which they were assigned, either the physiotherapy group or the placebo one. They were informed about the difference between the acupuncture groups. The control group was the group that received the placebo care.

2.3. Statistical analysis

All data were analyzed using SPSS 16.00 software (IBM Software,

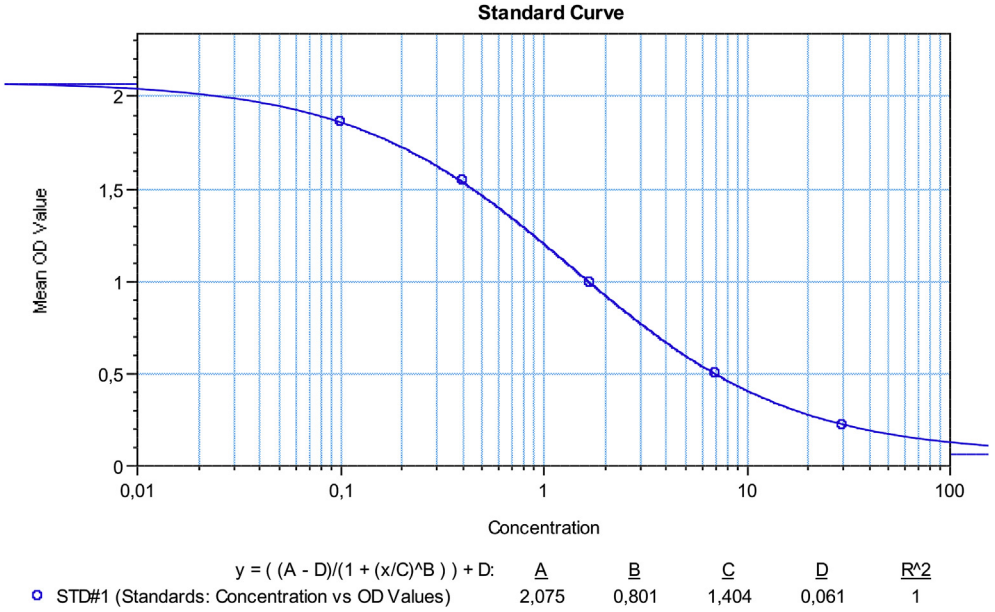

Fig. 1. A standard curve was drawn by plotting the mean cortisol absorbance value obtained from each standard sample against its concentration, with absorbance value on the vertical (y) axis and cortisol concentration on the horizontal (x) axis.

Table 2
Baseline demographics and pain characteristics measured by the chronic pain grade (CPG) scale and salivary cortisol concentration (SCC) data (mean ± SD).

	Group A (n = 10)		Group B (n = 10)		Group C (n = 10)	
	pre	post	pre	post	pre	post
CPG						
Pain intensity (Grades 0–5)	2.20 ± 0.51	1.90 ± 0.30	2.40 ± 0.51	0.60 ± 0.69	2.40 ± 0.51	1.10 ± 0.31
Disability score (Grades 0–5)	2.70 ± 0.48	2.00 ± 0.61	1.60 ± 0.64	0.50 ± 0.70	2.00 ± 0.81	0.70 ± 0.48
Disability days (Points 0–3)	1.00 ± 1.63	0.00 ± 0.00	0.70 ± 0.67	0.00 ± 0.00	0.50 ± 0.97	0.00 ± 0.00
SCC (ng/mL)	3.90 ± 3.15	1.67 ± 1.95	2.60 ± 2.68	0.88 ± 0.72	1.35 ± 0.95	0.91 ± 1.05

Group A = physiotherapy group, Group B = acupuncture group, Group C = control group.

Armonk, NY). Tests for normality were performed by the Kolmogorov-Smirnov test. Between-groups effect size and data homogeneity were assessed with repeated-measures analysis of variance (ANOVA) by Eta square and Levene's test, respectively. Mean values and standard deviations (SD) were calculated for pain levels, and salivary cortisol concentration (SCC) was obtained for each patient before and after treatment across the three groups. Frequencies were calculated from the topographic distribution of pain to record the musculoskeletal pain areas. Repeated-measures ANOVA (groups A, B, and C by time [pre, post]) were applied to test for group differences in pain levels (pain intensity and disability score) and cortisol levels, where the time factor had two levels (pre- and posttreatment protocol effect). A post hoc analysis was based on Tukey's HSD criterion, and Bonferroni adjustments were applied to determine the group differences in the pre- and posttreatment protocol effect. Results were considered statistically significant if p values were less than 0.05.

3. Results

The observed power for the variables pain intensity, disability score, and SCC was 1.00. Partial Eta square was 0.951 (95.1%) for pain intensity, 0.890 (89%) for disability score, and 0.603 (60%) for the SCC, with a significant level of less than 0.05. Hence, effect size analysis was around the medium and large effect. Levene's test showed that the population variances were equal for all groups at pre- and posttreatment effect on each variable.

Pre- and posttreatment pain levels and SCCs are summarized in Table 2. The characteristics of pain intensity ranged from grade I to II (≥50) at pretest and from grade I to 0 (<50) at posttest condition across the three groups. The disability score ranged from grade I (low disability <3 points) to grade III (high disability 3–4 disability points) at pretest and to grade I (low disability <3 points) on posttest across the three groups. The disability days ranged from 1 (7–14 days) to 0 (0–6 days) points across the groups, either at pre- or posttest condition across the three groups.

Analysis of topographic distribution of pain revealed that back pain was the most frequent musculoskeletal pain of the three groups (Table 3).

Repeated-measures ANOVA showed statistically significant differences in pain intensity both within the groups ($F = 27.15$, Sig = 0.00, $P < 0.05$) and between groups ($F = 4.22$, S = 0.02, $P < 0.05$). The percentage of true variance over total variance was $\omega^2 = 56.5\%$. Thus, ω^2 indicated that 56.5% of the total variance was accounted for by the treatments for the dependent variable pain intensity on posttest. Tukey's HSD post hoc analysis determined that the above significant differences originated from experimental group B (acupuncture treatment) in comparison with experimental group A (physiotherapy treatment; D = 0.55, S = 0.01, $P < 0.05$; Table 4). There were no statistically significant differences between the experimental groups and the control group (sham treatment).

In addition, repeated-measures ANOVA showed statistically significant differences in disability score between the groups ($F = 13.48$, S = 0.00, $P < 0.05$). The percentage of true variance over total variance was $\omega^2 = 58.6\%$. Thus, ω^2 indicated that 58.6% of the total variance was accounted for by the treatments for the dependent variable disability score on posttest. Tukey's HSD post hoc analysis determined that the above results were significant in experimental group B (acupuncture treatment) compared with experimental group A (physiotherapy treatment; D = 1.30, S = 0.00, $P < 0.05$), as well as for experimental group A compared with control group C (sham treatment; D = 1.00, S = 0.00, $P < 0.05$; Table 4). There were no statistically significant differences between experimental group B and control group C.

Table 3
Topographic distribution of musculoskeletal pain areas and pain level data for the three groups.

Musculoskeletal pain area	Group A (n = 10)		Group B (n = 10)		Group C (n = 10)	
Spine	*n*	%	*n*	%	*n*	%
Neck	4	40%	3	30%	1	10%
Thorax	1	10%	2	20%	4	40%
Back	8	80%	9	90%	7	70%
Pelvic	1	10%	0	0%	4	40%
Upper Extremity						
Shoulder	1	10%	9	90%	3	30%
Elbow	2	20%	1	10%	2	20%
Wrist	2	20%	1	10%	4	40%
Hip	4	40%	5	50%	0	0%
Lower Extremity						
Knee	7	70%	1	10%	3	30%
Ankle	0	0%	0	0%	2	20%
Chronic Grade Pain (CGP)/group						
Pain Intensity (Grades 0–5)	2.20 ± 0.51		2.40 ± 0.51		2.40 ± 0.51	
Disability score (Grades 0–5)	2.70 ± 0.48		1.60 ± 0.64		2.00 ± 0.81	
Disability days (Points 0–3)	1.00 ± 1.63		0.70 ± 0.67		0.50 ± 0.97	

Group A = physiotherapy group, Group B = acupuncture group, Group C = control group.

Table 4
Pain intensity and disability score data at pre- and posttreatment for the three groups.

Variable	Mean	SE	95% CI	Within-subject comparison	Between-subject comparison	Post hoc (Tukey's HSD)
Pain intensity (pre/−posttest)	1.76	0.07	1.60–1.92	$F = 27.15$ $P = 0.00^*$	$F = 4.22$ $P = 0.02^*$	**Groups: B-A** D = 0.55 $P = 0.01^*$
Disability score (pre/- posttest)	1.58	0.10	136–1.80	$F = 2.7$ $P = 0.08$	$F = 13.48$ $P = 0.00^*$	**Groups: B-A** D = 1.30 $P = 0.00^*$ **Groups: A-C** D = 1.00 $P = 0.00^*$

SE = standard error, 95% CI = confidence interval, * = statistically significant, D = mean difference, Group A = physiotherapy group, Group B = acupuncture group, Group C = control group.

Regarding the SCC data, repeated-measures ANOVA showed statistically significant differences both within groups ($F = 12.79$, Sig. = 0.00, $P < 0.05$) and between groups ($F = 41.06$, S = 0.00, $P < 0.05$). The percentage of true variance over total variance was $\omega^2 = 72.7\%$. Thus, ω^2 indicated that 72.7% of the total variance was accounted for by the treatments for the dependent variable SCC on posttest. Tukey's HSD post hoc analysis revealed that there were no statistical differences across the three groups. The total SCC across the three groups ranged from 2.62 ± 2.59 (ng/mL) at pretreatment to 1.15 ± 1.35 (ng/mL) at posttreatment. Bonferroni adjustments in pairwise comparisons for the three groups showed that the mean SCC levels decreased significantly at posttest compared with pretest condition (mean difference 1.46, Sig. 0.001, $P < 0.05$; Table 5; Fig. 2).

4. Discussion

The objectives of this prospective feasibility study were to determine the effect of two treatment regimens—physiotherapy or acupuncture—in comparison with sham therapy on a) pain intensity and disability and b) saliva cortisol levels in patients with CMD. The results of this study revealed a significant decrease in pain intensity, disability, and saliva cortisol levels posttreatment compared with the pretreatment values in patients with CMD. It can be postulated that these significant changes were the result of the treatments and accounted for a moderate proportion of the variance ($\omega^2 = 56.5\%$) for the dependent variables pain intensity, the disability score ($\omega^2 = 58.6\%$), and the SCC ($\omega^2 = 72.7\%$) at posttest. These estimations imply that the treatment regimens used in this study could be considered as effective treatment.

Topographic distribution of musculoskeletal pain areas reported by patients in each group showed that back pain was the most frequent musculoskeletal pain in the three groups. Most of the patients of the three groups reported more than one painful musculoskeletal area.

Previous studies have confirmed that back pain is the most common musculoskeletal disorder in Western societies (Rubinstein et al., 2010; Schell et al., 2008; Manec and MacGregor, 2005; Geiss et al., 1999). In addition, the patients treated with acupuncture experienced a statistically significant decrease in pain intensity compared with the physiotherapy group posttreatment. Our findings are in agreement with those of previous studies that have shown that acupuncture is a particularly effective treatment regimen for musculoskeletal pain management compared with other treatment regimens in patients with chronic back pain (Vazouez-Mejuto et al., 2014; Hutchinson et al., 2012; Rubinstein et al., 2010; Cherkin et al., 2009; Sherman et al., 2009) neck, shoulder and knee pains (Fu et al., 2014; Kim et al., 2013; DeBar et al., 2011; Schell et al., 2008).

Table 5
Salivary cortisol concentration data at pre- and posttreatment for the three groups (Bonferroni pairwise comparisons).

SCC	Mean	SE	95% CI	Significance
Pretest	2.62	0.44	1.70–3.54	
Posttest	1.16	0.24	0.65–1.66	
Mean difference (ng/mL) (pre-/posttest)	1.46	0.40	0.62–2.30	$P = 0.00^*$

SCC = salivary cortisol concentration, SE = standard error, 95% CI = confidence interval, * = statistically significant, Group A = physiotherapy group, Group B = acupuncture group, Group C = control group.

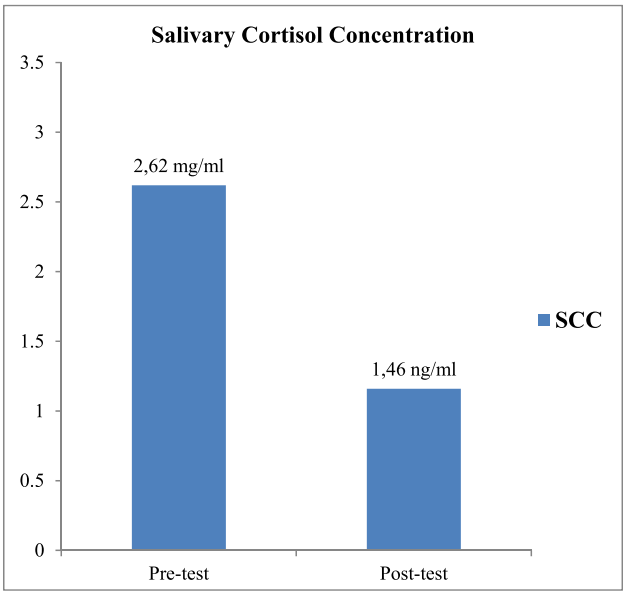

Fig. 2. Bonferroni pairwise comparisons showed the mean SCC levels were significantly decreased at posttest compared with those at pretest for the three groups (mean difference = 1.46, Sig. = 0.001, $P < 0.05$). SCC: salivary cortisol concentration.

However, it should be noted that there were no statistically significant differences in pain intensity between the experimental groups and the control group (sham treatment) posttreatment. Nevertheless, sham or placebo treatments have been reported to exhibit clinically significant improvements in quality of life in patients with multiple chronic problems (Benedetti et al., 2011). Thus, further studies with longer follow-up periods are required for more conclusive results.

In this study, we also observed a significant decrease in pain disability in both experimental groups compared with the control group posttreatment. The greater difference resulted from acupuncture treatment, and this was considered clinically significant. However, it is still unclear whether acupuncture provides a physiologically important stimulation or represents a placebo effect, and this treatment regimen remains unsubstantiated even if it reduces pain (Penney et al., 2016; Sherman et al., 2009; Cherkin et al., 2009; Vas et al., 2005).

In addition, the disability/painful period that resulted in 0–14 absences from the patients' daily activities, in either the pre- or

posttreatment condition, was not considered because all patients were characterized as having an active level of life, and we assumed their musculoskeletal painful areas to be both symptomatic and nonsymptomatic. Thus, our results mainly support the effectiveness of the acupuncture regimen on pain management in terms of both pain intensity and disability in patients with CMD.

With regard to the SCC, our findings showed that the total mean values across the three groups ranged from 3 to 5 ng/mL at pretreatment and from 1.5 to 3 ng/mL at posttreatment, thus exhibiting an almost 50% reduction.

Even though decreases in SCC were evident on posttreatment within groups and between groups, statistically significant differences were not found between the three groups. However, Bonferroni adjustments showed that the mean difference in SCC significantly decreased at posttest compared with pretest in pairwise comparisons for the three groups.

Our findings showed that the different treatment conditions (i.e., physiotherapy, acupuncture, or sham therapy) had similar effects on the decrease in SCC in all patients.

The findings of previous related studies regarding the effects of different therapeutic regimens on saliva cortisol levels in this population are controversial. Some studies found increased salivary cortisol levels to be associated with higher pain severity scores among subjects with chronic musculoskeletal pain conditions (Neeck, 2000; Neeck and Riedel, 1999). Other studies showed decreased cortisol levels to be associated with higher pain severity among healthy controls and patients with chronic pain (Goodin et al., 2012). Most of these studies assessed the cortisol levels in an attempt to identify the best doses and patterns of treatment (Carnes et al., 2007). On the other hand, systematic reviews have mainly evaluated the effect of multiple therapeutic methods on pain relief in chronic musculoskeletal pain (Mason et al., 2004). Clinical salivary cortisol levels have also been assessed from the perspective of the HPA axis function or central pain mechanisms in patients with CMD. Specifically, some clinical studies focusing on patients with fibromyalgia found hyperactive HPA axis responses and cortisol diurnal variation (Generaal et al., 2014; McBeth et al., 2005). However, more studies are needed to fully elucidate the effectiveness of therapeutic regimens on salivary cortisol levels.

Overall, our work might be considered as the first evidence of the effectiveness of different therapeutic regimens on the regulation of SCC levels and their relation to pain and disability levels in patients with CMD.

4.1. Limitations

Overall, our work might be considered as a point of reference for future studies on the effectiveness of the treatment regimens used in the present study. However, these results should be interpreted with caution because of some potential limitations of the study. Specifically, the size of the study sample was limited for various reasons (e.g., a wide variety of pain locations in the patients that made the classification of the CMD patients difficult at baseline). Thus, this study should be considered as a prospective feasibility study. Future studies should focus on more specific pain disorders/locations.

Moreover, it should be noted that the study sample consisted of both male and female participants, and this could affect the accuracy of our results because of potential gender differences in pain and emotional responses as well as cortisol concentrations in stress conditions.

In addition, the patients included in the physiotherapy and control group were treated by the same clinician, and this could limit the reliability of our results because of the lack of a blind procedure.

Moreover, in physiotherapy practice, there is no specific treatment description for managing patients suffering from musculoskeletal pain disorders in different body sites. This could be a bias affecting the reliability of our posttreatment results, and future studies should focus on the implementation of evidence-based therapeutic interventions. To accomplish this, there is a need to select, adapt, and evaluate intervention studies, with the attempt to classify specific treatment regimens in different groups of musculoskeletal patients. The history of pain is commonly a difficult factor to measure in musculoskeletal pain disorders, and the lack of this information could also be a limitation of this study.

In addition, various confounding factors can affect cortisol levels, such as food habits, daily circadian rhythm, sex, activities, habits, and previous history of trauma. Further research should control as many of the above factors as possible so that more conclusive findings can be revealed.

5. Conclusion

The results of this study revealed a significant decrease in both pain and cortisol outcomes in patients with CMD as a result of posttreatment regimens used. Decreases in pain intensity and disability levels were mainly identified in the experimental group treated with acupuncture. No statistically significant differences were found between the three groups in salivary cortisol levels, although significant differences (decreases) were observed in pairwise comparisons for the three groups posttreatment.

Thus, we cannot draw conclusions regarding whether the reduction in SCC could be an indication of pain reduction in patients with CMD. First, we should be careful in interpreting the clinical effect of our results because of cortisol-related confounding factors, the limitations of this study, and the various results reported in previous studies. Second, a prospective feasibility study design was applied because of the need to select, adapt, and evaluate the effect of different treatment regimens on our outcomes measures. Nevertheless, investigators can eventually use these data to further determine whether the associations between musculoskeletal pain, SCC, and treatment regimens are justified in patients with CMD.

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Contribution of each author

1. Substantial contribution to the study conception and design, data acquisition, analysis, and interpretation: Maria Papandreou, Anastassios Philippou, and Orjona Taso.
2. Substantial contribution to physiotherapy and acupuncture sessions: Maria Papandreou and Alexandra Kapedra.
3. Drafting or revising the article for intellectual content: Maria Papandreou and Anastassios Philippou.
4. Agreement to be accountable for all aspects of the work related to the accuracy or integrity of any part of the work: Maria Papandreou, Anastassios Philippou, Orjona Taso, Michael Koutsilieris, and Alexandra Kapedra.
5. Approval of the final version: Maria Papandreou, Anastassios Philippou, Orjona Taso, Michael Koutsilieris, and Alexandra Kapedra.

Declaration of competing interest

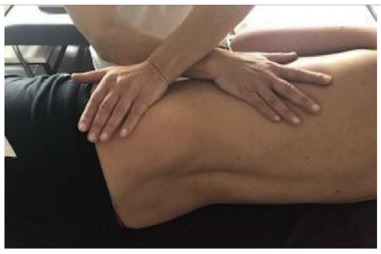
The corresponding author of this study, Dr. Maria Papandreou, and all the co-authors declare no conflicts of interests.

Appendix

Treatment protocols performed by the physiotherapist in experimental group A:

A. Spine pain: low-back pain issues, thorax, pelvic imbalances.

General information of myofascial treatment release (MTR): Based on sinking techniques using cross- or parallel hand placement for 10–20 min maximum *(Ajimsha* et al., *2015; McKenney* et al., *2013*, *Schleip* et al. *2012*, *Stecco* et al. *2007*, *2008)*.

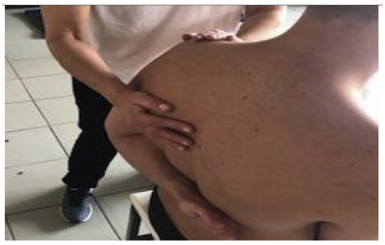
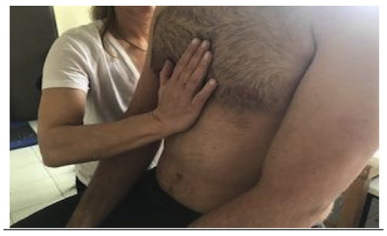
Exercise	Demonstration
MRT on back extensor muscles: In this technique, the patient lies prone. One hand is placed on the sacrum and the other hand on the lumbar spine. The therapist gently presses the tissue (on the posterior superficial and deep anatomical regions of fascia) and waits for the release for approximately 10 min or more.	
MTR on quadratus lumborum: In this technique, the patient lies on his or her side. A pillow may be placed under the contralateral side, and the uppermost lower limb is extended and in line with the body, while the other lower limb is flexed at the knee. One hand is placed on the 12th rib and the other hand on the iliac spine of the lateral side. The therapist applies a sustained gentle downward pressure (on the lateral superficial and deep anatomical regions of fascia) for 10 min or more and waits to yield sensation.	

Stretching and strengthening exercises.

Targeted Muscles	Sets and Repetitions
Quadratus lumborum, hip adductor, and hamstring muscles	Stretching exercises 3–5 reps × 30 s
Double knee-to-chest exercise with the assistance of both hands	10 repetitions
Lower limbs and trunk rotation with feet resting on the table, while rotating the head to other direction	10 repetitions in each direction
Anterior and posterior pelvic tilts performed in supine (emphasis on neutral position)	15 repetitions
Single knee-to-chest exercise with the assistance of both hands	10 repetitions for each limb.
Transervus abominis and multifidus muscle strengthening in supine and prone positions	2 sets × 15 reps (10-s isometric contraction)

(Gatti et al., 2011).

B. Upper limb pain: Neck, shoulder, elbow, and wrist pain.

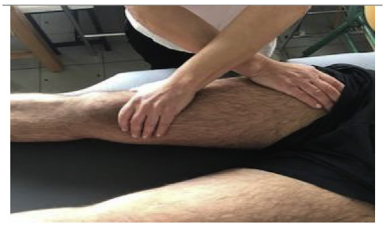
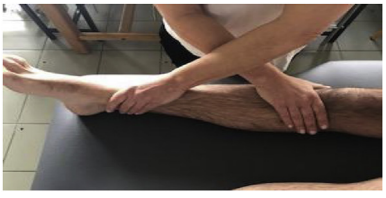
Exercise	Demonstration
MTR for shoulder and neck pain and dysfunction: In this technique, the patient is in the side-lying or seated position with thoracic and cervical spine in the neutral position. The arm is placed behind the back into internal rotation. The physiotherapist stands at one side of the patient; he or she places one hand at the back and cups the shoulder and inferior angle of the scapula. Then, the physiotherapist gently lifts the patient's shoulder to the ceiling and waits for the sense of yielding. The physiotherapist applies a sustained gentle upward pressure (on the anterior superficial, deep arm, and superficial back anatomical fascia lines) for 10 min or more and waits to yield sensation.	
MTR on thorax: The patient sits in a relaxed/neutral position, and the physiotherapist places his or her hands between the scapula and the sternum. The physiotherapist applies a sustained gentle downward and upward pressure for 10 min or more, following inhalation and exhalation, and waits for a yielding sensation.	

Stretching and strengthening exercises.

Targeted Muscles	Sets and Repetitions
Neck, thorax flexors, and extensor muscles stretching exercise	Stretching exercises 3–5 reps × 30 s
Shoulder external/internal rotator muscles, major thoracic muscle, and anterior/posterior capsule stretching exercises	Stretching exercises 3–5 reps × 30 s
Strengthening exercises: Neck flexion/extension active range-of-motion exercise in seated position using 1 finger between the chin and sternum for the right amount of movements	10 repetitions
Shoulder exercises in supine, seating, and prone position:	2 sets of 10–15 reps with 10-s isometric contraction
Supraspinatus muscle in full can position, internal/external rotator muscles at 0° abduction, lower/middle trapezius prone full can and prone row	

(Reinold et al., 2009).

C. Lower limb pain: Hip, anterior thigh pain, knee dysfunction, and pain structures of the lower leg and ankle.

Exercise	Demonstration
MTR on suprapatellar. In this technique, the patient lies supine. One hand is placed on the superior edge of the patella at the lower part of the rectus femoris of the knee and the other hand at the upper part of rectus femoris of the hip, with the hand crossed (on the front superficial anatomical region of fascia), having contact with the anterior part of the femur. The physiotherapist applies a sustained gentle pressure for 10 min or more and waits to yield sensation.	
MTR on infrapatellar: In this technique, the patient lies supine. The physiotherapist places one hand on the inferior edge of the patella and the other hand on the anatomical area between the malleolus of the ankle, with the hands crossed (on the front superficial anatomical region of fascia), having contact with the medial tissues of the anterior compartment of the lower leg. The physiotherapist applies a sustained gentle pressure for 10 min or more and waits to yield sensation.	

Strengthening-stretching exercises.

Quadriceps, hamstrings, soleus, gastrocnemius, and iliotibial band	Stretches for 3–5 reps, for 30 s with maximum range of motion as far as the patient can tolerate
Straight leg raise in supine	2 sets × 20 reps
Isometric hip abduction, external/lateral hip rotation, and hip extension exercises	2 sets of 20 reps with 5-s isometric contraction in supine and prone positions
Dorsiflexion/plantarflexion of the ankle (up and down) exercises on standing position	2 sets × 12 reps

(De Marche Baldon Rodrigo et al., 2014).

Treatment protocols performed by the doctor-acupuncturist in experimental group B:

General information: The acupuncturist doctor used sterile disposable needles (0.25 mm × 0.25 mm) at least 1.5 inches in length. Needling depth varied slightly, depending on the acupuncture point, but it was generally between 1 and 3 cm. This was the treatment prescribed by the doctor at the beginning of each visit. It could include any acupuncture points that could be needled with the participant lying prone or supine by using 5 to 10 averaged needles for 45 min (30 min with stimulation by twirling the needles at 10 min just prior to needle removal) at maximum real time. The acupuncturist manipulated the needles to elicit qi, which they perceive as a biomechanical response in tissue *(Fu* et al., *2014*; *Vazouez-Mejuto* et al., *2014*; *Cheshire* et al., *2013*; *Kim* et al., *2013*; *DeBar* et al., *2011*; *Hutchinson* et al., *2012*; *Cherkin* et al., *2009)*.

1) **Standardized acupuncture for spine/back pain:** This included at least 8 acupuncture points: Du Mai 3, BL 23, 52, 40, KI 3 bilaterally *(Fu* et al. *2014*; *Vazouez-Mejuto* et al. *2014*; *Cheshire* et al. *2013*; *Kim* et al. *2013*; *DeBar* et al. *2011*; *Hutchinson* et al. *2012*; *Cherkin* et al. *2009)*.
2) **Standardized acupuncture for lower limb/knee pain** (*Kim* et al. *2013)*: This included at least 9 acupuncture points: SP9, GB34, ST36, ST35, EX-LE5 (Xiyan), BL60, GB39, SP6, and KI3.
3) **Standardized acupuncture for upper limb/shoulder/neck pain** *(Jorge* et al. *2005; Qing-Nan Fu et al., 2014):* This included local and distal acupuncture points: local: LI15, TE14, SI9, LI14; distal: ST38, GB34, BL57

SP = spleen meridian, GB = gold blander meridian, ST = stomach meridian, EX-LE = extra points, BL = bladder meridian, KI = kidney

meridian, LI = large intestine meridian, SI = small intestine meridian, TE = triple warmer meridian.

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