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THE IMPACT OF MANUAL THERAPY TECHNIQUES ON PAIN, DISABILITY AND IL-1B LEVELS IN PATIENTS WITH CHRONIC CERVICAL PAIN

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ABSTRACT

Background: Chronic neck pain is a common musculoskeletal dysfunction, and Manual Therapy is one of the effective treatment modalities, but underlying mechanisms of action are unclear. The study's purpose was to investigate the short-term effect of mobilization-manipulations on patient's symptoms and to detect changes in the concentration of inflammatory biomarker interleukin-1b.

Methods: Twenty-two patients aged 20-50 years with chronic neck pain, randomly assigned into two groups. The study group received nine sessions of a three-week Manual Therapy intervention. The control group received SHAM Manual Therapy of the same dosage. Pain and functionality measures conducted before and after the intervention via NPRS and NDI scales, respectively. Blood samples collected at baseline, after the first session and post-intervention, detecting IL-1β concentration, using the corresponding ELISA kit. Mixed ANOVA statistical analysis implemented for differences in the GROUP-TIME factors.

Results: There was a significant statistical interaction between factors and significant main effects on pain, functionality, and IL-1β ($p < .05$). IL-1β was statistically significant reduced ($p < .05$) at second measurement for the study group, but not significantly reduced ($p > .05$) between second and final measurement. No statistical significance was found for the control group on any of the dependent variables across measures.

Conclusion: Patients with chronic mechanical pain showed significant pain and functionality improvement after manual therapy application. The underlying mechanism of action seems to relate with a reduced IL-1b concentration of reinforcing future research at relevant pain biomarkers.

Keywords: manual therapy, chronic neck pain, cytokines.

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INTRODUCTION

Chronic mechanical neck pain (CNP) is the clinical condition of pain for at least twelve weeks, associated with painful movement of cervical spine (Guzman et al., 2008), distinguished from non-mechanical pain as radiculitis, whiplash injury, headache, spondyloarthropathy, rheumatic arthritis [1], [2], [3]. CNP has a 12-month prevalence between 10% and 20% [4]. Predisposing factors are repetitive movements, lack of interruptions, static work with the head and upper limbs in the same position for longer times [5]. The exact mechanism of pain is still unclear and probably facilitation of afferents stimuli located at the articular surfaces and soft tissue or even at the Luschka intra-articular interact at the vertebral level A3-A7 [6].

Manual Therapy (MT), as a conservative treatment approach for CNP patients, seams to improve symptoms, increasing range of motion, and reducing pain levels [7]. The latest systematic reviews and guidelines recommend MT implementation in either the form of manipulation (High-Velocity Low Amplitude-HVLA) or mobilization (Low-Velocity Low Amplitude-LVLA), based on the moderate quality of evidence [8]. Prognostic factors of MT response to treatment are related to baseline Neck Disability Index (NDI) score <11.5, unilateral symptom localization, lack of radiculitis signs, and lack of pain in cervical spine extension [9].

The underlying mechanisms by which these techniques influence the physical history of cervical dysfunction remains elusive. This biomechanical model (positional fault hypothesis), which supports the reposition of intra-articular surfaces, has not been proved yet. The more updated neurophysiological approach is characterized by direct stimulation on cervical spinal synovial motor receptors via MT application and indirect inhibitory effect on nociceptors [10], [11]. At the presynaptic level, there is an activation of the endogenous opioid system and downward pain inhibitory system [12]. Also, central sensitization, which results in the low degree of inflammation that characterizes chronic musculoskeletal disorders, is alleviated by modifying mechanical and chemical stimuli after neck manipulations application (13],[14].

Pain, as a symptom of subjective evaluation, needs to objectively measure in accordance with pain biomarkers (noci-markers) [15]. Measuring detectable chemical pain signals in CNP patients' blood as it is cytokines, a possible statistically significant reduction is expected after MT intervention, combined with a corresponding decrease in pain levels. Therefore, the study hypothesis refers to the possible reduction of cytokine levels after MT application combined with symptoms improvements.

It has been shown that different mechanical stimuli in terms of degree, direction and type of force applied (traction, compression, shear, flexion, bend) at the cellular level, induces fibroblasts responses and extracellular/intercellular chemical changes [16]. [17].

Each vertebral segment comprises a number of mechanoreceptors, nociceptors, pressure, and temperature receptors, which interact with internal and external stimuli [18]. Motor-neurons stimulation induces analgesic effect via posterior spinal horn, facilitating a downward pain system and increasing endorphins concentration [19]. Even more, MT application decreases the intra-articular producing reflex paraspinal muscle relaxation and ROM increase [20, 21]. Immune system cells (neutrophils and macrophages) of the respiratory system, has been shown to increase in peripheral blood after thoracic manipulation, without significant changes in substance P [22].

Clinical and experimental observations have correlated IL-1b with inflammatory musculoskeletal disorders [23]. Concentrations of IL-1β show a strong correlation with IL-6 and IL-1Ra receptor antagonist ($p < 0.0001$), a particularly important observation, since the treatment of patients with IL-1β antagonists reveals a corresponding decrease in IL-6 [23]. Age and gender factors influence cytokine values since concentrations of IL-6, and IL-1Ra increase in the elderly, and also significantly higher concentrations of IL-1Ra have been identified in women [2]. We decided to examine IL-1β, due to clinical association with pain in chronic musculoskeletal disorders but also because of the possibility of detecting changes in peripheral blood [25], [26]. Therefore, the primary study's purpose was to examine the short-term effects on pain and functional levels following the application of Manual Therapy techniques in CNP patients. The secondary aim was to investigate the underlying mechanism of action in inflammatory biomarker concentration values and particular pro-inflammatory cytokine IL-1β.

METHODOLOGY

The study designed under a parallel-group double-blind, randomized controlled clinical trial. The double-blinded sampling undertaken by two researchers. One responsible for measurement conduction and the other for MT techniques implementation. The study sample consisted of 22 patients with chronic mechanical neck pain divided into two groups of 11 subjects. Patients presented with a physiotherapy referral from an orthopedist or neurologist physician. A cooperating microbiological laboratory conducted the planned blood sampling. The data collection period lasted from April to August 2018.

The patient's age ranged from 20-50 years and was associated with increased frequency of mechanical pain, limiting the effect of age over cytokine variation [27], [28]. Potential confounding factors that could influence measurements outcome such as physical activity, dietary habits, medication intake were considered in terms of demographic and lifestyle data collection questionnaires.

The study conducted in a private physiotherapy clinic under collaboration with the Laboratory of Experimental Physiology of the Department of Medicine of the National and Kapodistrian University of Athens, which was in charge of the research program.

Inclusion criteria consisted of patients aged 20-50 years old, with mechanical pain in the cervical spine to the first thoracic vertebra, for at least three months and a score of 2 points on the Numerical Pain Rating Scale (NPRS) and

20% on Neck Disability Index (NDI).

Exclusion criteria consisted of cervical pain with radiculitis, pain or numbness in the upper extremities, whiplash injury, myelopathy, neoplasia, rheumatoid arthritis, metabolic disease, history of surgical surgery, signs dizziness, vertigo and/or headaches, corticosteroid and/or NSAIDs usage for the last six months [3]. They should also be no smokers or highly exercised active, avoiding potential effects on cytokine levels [29].

Participant's enrollment completed after therapist evaluation, reproducing pain in the examined cervical spine through techniques like Joint-play, end-feel, and provocation-alleviation tests [30], [31].

Patients were randomly assigned to one of the two groups, study, and control. Group allocation conducted using a random numbers computer program [32]. Participants evenly distributed to equal groups and before the intervention, they completed the consent-form. Moreover, they consulted to follow their regular daily activity and to avoid movements that would affect their symptoms. No instructions were given in relation to home-based therapeutic exercises.

Intervention protocol based on manual therapy techniques implemented so far on current evidence, as well as on daily clinical experience. The study group received a three-week duration of mobilization and manipulation application, consisted of nine sessions in a frequency of three per week. Passive traction mobilization (Low Volume Low Amplitude-LVLA) was applied on a seated and supine position at the level of the disc joints, and bilateral manipulation (High Volume Low Amplitude-HVLA) performed in the supine position at thoracic vertebrae level of T1-T8 facet joints. Research has shown that thoracic manipulation is effective in conjunction with LVLA techniques for further improvement on neck pain patients [3], [33], [34].

Audible click production was not a criterion for successful implementation [35]. The thoracic spine as an area of intervention was chosen due to regional interdependence with the cervical spine [36], [37]. Guidelines on thoracic mobilization as evidence-based therapy in CNP patients have been incorporated [8]. Furthermore, cervical manipulation is not superior to thoracic manipulation in these patients, and not free of complications (38], [39], [40].

Specific mobilization was selected (application to specific vertebral levels according to symptoms and clinical examination) and not generalized, despite the evidence ambiguity in distinguishing their therapeutic effect on chronic cervical pain [41], [42]. It has been shown that 70% of patients with bilateral symptoms also respond to generalized mobilization [43].

The control group followed the same intervention protocol but in virtual form (SHAM Manual Therapy) of the same duration and frequency. Direction and degree of force were absent, with the therapist simply touching his hands with the neck of the patient, from the same starting position, without performing any manual technique. SHAM Manual Therapy techniques can be included in a research program as a placebo application, taking into account the principles of bioethics, but also that simple contact with the patient is likely to affect the measurements [44]. The patient's inclusion criterion of non-participating in rehabilitation programs through manual therapy techniques before the present study strengthened their difficulty in recognizing therapeutic (MT) and virtual (SHAM MT) applications [44], [3].

Study measurements included cytokine IL-1b concentration blood levels over three-time phases: before the intervention, after the first session, and at the end of the program. Detection of IL-1b in peripheral blood based on research data, regarding the sensitivity of concentration changes to chronic musculoskeletal pain. We chose to implement the second IL-1b measurement after completion of the first treatment session, due to potential acute effect of MT. Pain and Disability were measured before and after intervention using Numeric Pain Rating Scale (NPRS) and Neck Disability Index (NDI) respectively, as the most common research tools of these CNP patients [45], [46]. Both questionnaires are reliable and valid in measuring symptoms of patients with chronic cervical pain (NPRS ICC = 0.76), NDI (ICC = 0.86–0.98), [46]. NPRS has a minimal detectable change (MDC) of 2.1 and a minimal clinically significant difference (MCID) of 1.3 in patients with mechanical neck pain [46]. NDI has an MDC of 20% or 6.9 and an MCID of 14% or 5.5 [45], [46].

Blood samples were taken between 3.30-4.30 pm. Patients remained seated and rested for at least 30 min, and 10 ml of blood was taken from the medial (oblique) vein of the forearm. Blood was placed in a glass vial for serum separation and left at room temperature for about 30 min. Samples were then centrifuged in a cooled centrifuge at 40 C° at 4,000 rpm for 10 min and the separated serum was obtained. Samples were separated in smaller volumes (300 μl), maintained at -80 C° and thawed for analysis. All samples were analyzed simultaneously and in duplicate, and their mean was taken as the final value. The second and third blood samples were taken 20 minutes after the end of the first session and after the ninth session respectively. ELISA protocol: Concentration of IL-1β (pg/ml) was determined by the standard double-antibody ELISA method (sandwich type), using commercially available reagent according to manufacturers' instructions. Analyzes with this method are reliable and highly specialized. Samples were plated on 96-well plates (Costar) coated with a specific monoclonal antibody against the protein to be analyzed, as well as samples with standard concentrations. Subsequently, the protein bound to its specific antibody was detected by binding of a horseradish peroxidase conjugate substrate to a second polyclonal antibody against the protein previously assayed and conjugated to the samples. Chromatography of the existing peroxidase applied with a tetra-methyl-benzidine (TMB) substrate. The produced tint was measured on a multi-cell plate reader (Versamax, Molecular Devices, CA, USA) at 450 nm, and protein (hormone) concentrations were calculated using special software (SoftMax Pro software, Molecular Devices, CA, USA). The minimum reagent detectable limit used for IL-1β was 2 pg/ml.

STATISTICAL ANALYSIS

Data were analyzed using SPSS, 25 (SPSS Inc., Chicago, IL). As dependent variables were defined, the NPRS, NDI, and cytokine IL-1β. Independent Variables (Factors) were distinguished on the between-factor factor (GROUP) comprising the two-level Manual Therapy and SHAM Manual Therapy protocol and the Within-subject factor (TIME) representing repetitive measurements at two levels before and after intervention for NDI, NPRS. As for cytokine concentration, three levels of IL-1b were defined. Data were analyzed using Mixed Analysis Of Variance (Mixed-ANOVA), detecting possible interaction of factors on the dependent variables. Because of the within-subjects factor measured twice (before and after intervention) in the same sample of participants, Mixed-ANOVA is chosen, instead of 2-way ANOVA, including the statistical assumption of independence of participants' measurements. In a mixed-model, the Between-subjects factor can also be defined as a covariate.

Due to the small sample size, Mixed ANOVA assumptions were investigated. Dependent variables measured on a continuous level. Testing for normality of dependent variables variation for each level of the independent variable (GROUP / TIME) was tested with the Shapiro-Wilk test for small sample sizes (<50 samples). Kurtosis and skewness of distribution were tested in an acceptable range of -2 to +2 standard deviations (SD) from the mean. Levene's test tested the homogeneity of the variations in pain scores, disability, IL-1b among participants. The Box's M test was used to detect the equality of covariates between groups. For this test, the significance level was set at p <.01. Due to the third measurement of cytokine, an additional Mauchly's test of sphericity was performed to check for equality of covariations between all pairwise comparisons of the two groups. The main statistical purpose was to detect any interaction between the GROUP X TIME factors of the independent variable in each of the dependent variables. In case of significant (p <.05) interaction, it was pre-determined to examine the statistical significance of simple main effects statistical factors in NPRS, NDI, IL-1b scores, as well as post-hoc analysis of paired comparisons within measurements (TIME), using Bonferroni correction for multiple comparisons, reducing the probability of Type I error. Confidence Interval (CI) was set at 95% and statistical significance at p <.05.

RESULTS

Twenty-two (22) patients, with a mean age of 42 years and mean symptoms duration of 50 months, met the inclusion criteria and randomly assigned to study and control groups. Demographics such as gender, age, body weight, height, and duration of symptoms are shown in Table 1. All patients completed the three (3) week manual therapy intervention protocol, consisting of nine (9) chiropractic sessions, as well as all measurements. There were any complication or loss of patients during the study. Descriptive statistics on NDI and NPRS, sample size (N), mean, and standard deviation (SD) are depicted in Tables 2-3.

Table 1: Demographics of participants (n) mean and standard deviation (± SD).

GROUP	STUDY	CONTROL
Sample, n	n=11	n=11
Gender-female, n	8	6
Age-years, mean, (SD)	40 (± 12)	44.7 (± 14)
Symptoms duration mean, (SD)	50 (±30.2)	32 (±58)
Weight-kg mean, (SD)	62.5 (±4.7)	72.2 (± 3.5)
Hight-cm mean, (SD)	166 (±6.)	171 (± 9)

Table 2: NPRS Descriptive statistics

NPRS	GROUP	Mean	Std. Deviation	N
NPRS1	1	4.9091	1.51357	11
	2	5.2727	1.90215	11
	Total	5.0909	1.68775	22
NPRS2	1	1.3636	1.62928	11
	2	4.6364	1.62928	11
	Total	3.0000	2.30940	22

Table 3: NDI descriptive statistics.

NDI	GROUP	Mean	Std. Deviation	N
NDI1	1	21.8182	7.23627	11
	2	21.4545	9.38471	11
	Total	21.6364	8.17980	22
NDI2	1	6.3636	5.98787	11
	2	25.0909	13.89572	11
	Total	15.7273	14.17302	22

NPSR, NDI Assumptions

Dependent variables of pain and disability measurements performed on continuous level scales, ranging from 0-11 and 0-50, respectively. There were no outliers. Box's M test was found not significant, p> .01 (p = .039, F = 2.798), assuming equality of variances of the NPRS variable between groups and also for NDI, p> .01 (p = 0.32, F = 2935). Levene's test was not significant, p> .05 for both variables so that we can conclude the equality and homogeneity variation of NPRS and NDI. Normality test for small Shapiro – Wilk test, was non-significant (p> .05), indicating the normal distribution of pain and disability values for each group of participants, as well as the symmetry (Skewness) and curvature (Kurtosis), which were within the acceptable standard deviation (SD) = -2 to +2 (George & Mallery, 2010).

Interaction

Mixed ANOVA analysis revealed statistically significant interaction (GROUP X TIME) for NPRS, F (2, 76) = 42.49, and NDI, F (2, 76) = 35.59, (p <.001), as depicted in non-parallel lines of profile plots (Figures 1, 2).

Figure 1: *Interaction of GROUP x TIME factors on NPRS.*

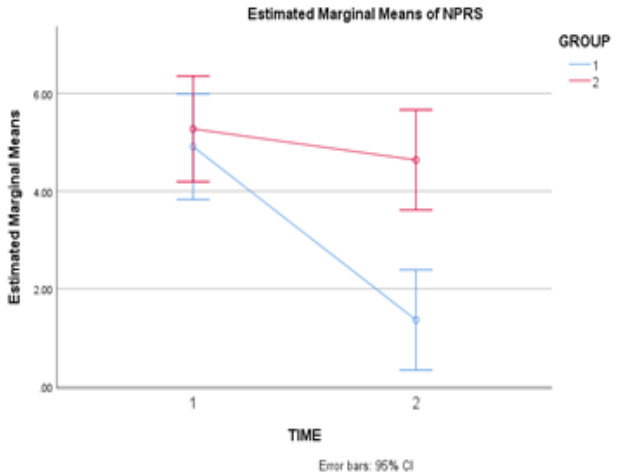
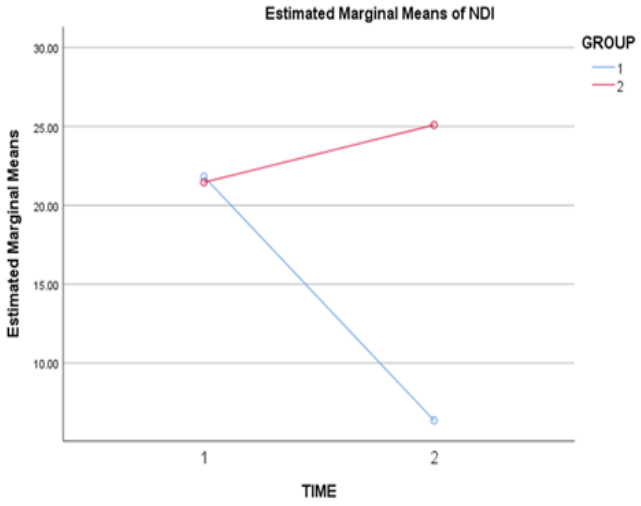


Figure 2: *Interaction of GROUP x TIME factors on NDI.*



NPRS Simple Main effect.

There was a significant main effect of the independent variable TIME (Within-subject effect) on the NPRS dependent variable for each level of the independent GROUP variable, F (1, 20) = 42.490, p = .000, (p <.05). There was a significant main effect of the independent variable GROUP (Between-subject effect) on the NPRS dependent variable for each level of the independent variable TIME F (1, 20) = 8.122, p = .01, (p <.05). Pairwise comparisons showed the significant statistical difference for the NPRS variable between groups before and after the intervention, p = .000, (p <.05), with Bonferroni correction.

NDI Simple Main Effect.

There was a significant main effect of the independent variable TIME (Within-Subject Effect) on the NDI dependent variable for each level of the independent GROUP variable, F (1, 20) = 8.895, p = .007, (p <.05). There was a significant main effect of the independent variable GROUP (Between-Subject Effect) on the NDI dependent variable for each level of the independent variable TIME F (1, 20) = 6.553, p = .019, (p <.05). This is also shown by pairwise comparisons, where a significant statistical difference was found for the NDI between pre- and post-intervention study and control groups, p = .007, (p <.05), with Bonferroni correction.

Biochemical examination of IL-1b.

Descriptive statistics for the two groups of patients regarding IL-1b values before intervention (ILPRE) after the first session (ILMID) and after the last session (ILPOST), are depicted in Table 4.

Table 4: Descriptive statistics for IL-1b.

	GROUP	Mean	SD	N
ILPRE	1	11.2549	6.32040	11
	2	7.2481	3.09878	11
	Total	9.2515	5.27257	22
ILMID	1	5.6180	3.11560	11
	2	7.0268	4.37595	11
	Total	6.3224	3.77634	22
ILPOST	1	4.2360	4.57138	11
	2	7.4543	2.99994	11
	Total	5.8451	4.11696	22

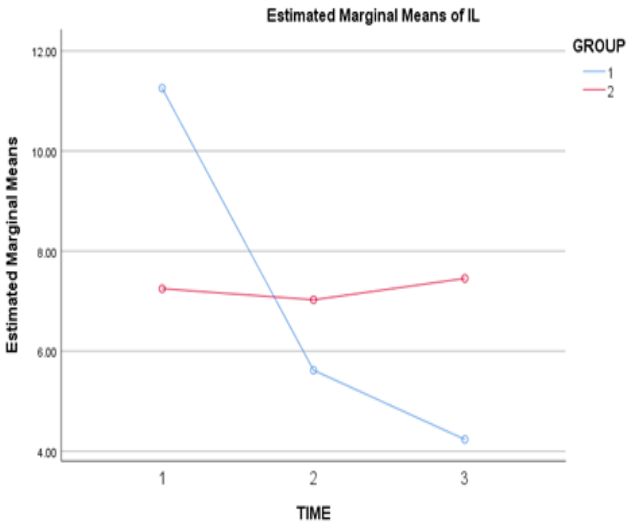
IL-1b Assumptions

Dependent variable IL-1b measured on continues level. Box's M test was found not significant, p> .01 (p = .021, F = 2.483), assuming equal co-variations of IL-1β between groups. Levene's test was found not significant, p> .05, so we can conclude that equality and homogeneity of IL-1β variations for the three measurements between the two groups. Normality Shapiro – Wilk test was found non-significant (p> .05), indicating the normal distribution of participants' IL-1β values. Sphericity assumed, via Mauchly's Test, which was non-statistically significant (p = .440), indicating equality of variances of all pairwise comparisons between the three measurements, avoiding the F-ratio error being inflated.

Interaction

Mixed ANOVA analysis showed a statistically significant interaction (GROUP X TIME) for the dependent variable IL-1β, F (2, 19) = 4.14 (p = 0.32, p <.05) (Figure 3).

Figure 3: Interaction of GROUP x TIME on IL-1β variable.



Simple Main Effect on IL-1β.

There was a significant main effect of the independent variable TIME (Within-Subject Effect) on the dependent variable IL-1β for each level of the independent variable GROUP, F (1, 20) = 5.129, p = .010, (p <.05). There was a significant main effect of the independent variable GROUP (Between-Subject Effect) on the dependent variable IL-1b for each level of the independent variable TIME F (1, 20) = 134.742, p = .001, (p <.05). Statistical differences between first ILPRE and the second ILMID measure were statistically significant, p = .013, (p <.05). The difference between the second ILMID and third ILPOST measures was not statistically significant, p = .657, (p> 0.5).

DISCUSSION

CNP patients represent one of the three-neck pain subgroups according to recent systematic reviews, with the other represent patients with upper extremities radiating pain and coordination Impairment disorders [8]. Source of pain is difficult to identify, as there are many anatomical structures implicated, such as facet, synovial connective tissue, muscle connections, intervertebral disc and neural tissue [47]. MT, according to moderate evidence-based, has a positive impact on cervical pain patients [48], [49]. MT induces relaxation stimuli on paraspinal muscles by reducing the CNS-induced neuronal potentials, reducing muscle spasm and pain [50]. Low excitation threshold motors appear to be activated through MT application techniques [51].

Identifying low-level inflammation biomarkers on peripheral blood such as the cytokines IL-1β, IL-6, TNF-a is a recent and quite promising approach to the underlying mechanisms of MT action in symptom improvements of CNP [16], [52]. Few studies applied a single session of MT to study the acute direct impact of these techniques [3]. In our study, this was desirable for the variable of IL-1b, as it has been shown that its concentration can change rapidly (20 minutes) after a single chiropractic stimulus (Teodorczyk et al., 2006). We found a statistically significant difference in the second measurement (decrease in IL-1β concentration) only in the study group, thus recording the immediate and acute effect of MT on biomarkers of inflammation. It was interesting that on the post-intervention measurement, the IL-1β concentration was not statistically lower compared to the second measurement, but only as a decreasing trend. Probably, the series of sessions applied, stabilizes biochemical profile, once this has been achieved at the start of the program. If further reductions can be achieved this may be revealed in a larger sample size research design and with an increased number of sessions. The control group showed any statistically significant difference between the three measurements, accounting for all dependent variables.

On musculoskeletal spine disorders, cytokine growth (TNF, IL-1b, IL-6) has been found, mainly in degenerated discs rather than in the intervertebral disc herniation patients, after culturing facet articular cartilage cells during surgery [53]. Repeated mechanical load in excess of tissue resistance affects the metabolism of cartilage cells, resulting in the production of cytokines and proteases [53]. Increased IL-1b in the facets was associated with increased pain [54]. Many factors determine the cytokine concentration like target cell, activation signal, timing, sequence of action and experimental model. Elevated IL-1β and MMP-1 levels were observed in patients with intervertebral foramen stenosis, but not in patients with intervertebral disc herniation. However, there was no significant difference in the value of TIMP-1 between the hernia and stenosis groups. Also, PCR results showed that MMP-1 mRNA in chondrocytes in vitro was dose- and time-dependent in response to IL-1β stimulation. Therefore, over-expression of MMP-1, induced by IL-1β, plays an essential role in the inflammatory process of degeneration on the vertebral joints [55].

Research has examined the MT effect on pain and functionality into a multifactorial rehabilitation program, such as exercise and/or thermo-electro-hydrotherapy [8]. However, designing the current study, we did not incorporate any other form of physiotherapeutic intervention to be more precise in the correlation between MT and changes in pain, functionality and cytokine levels. Our intervention protocol conducted in a series of sessions over three weeks, as this is the daily practice of rehabilitation programs to enhance the results generalizability and reproduction of the program by other researchers.

Up until now, study designs are distinguishable from those that applied laboratory MT stimulation on fibroblasts [56], [57]. Meltzer et al., 2010) and those of realistic MT application in patients [55], [61]. Teodorczyk (2006) found a decrease in TNFα, IL-1β production in asymptomatic subjects treated with single MT in the upper thoracic spine, before the intervention, after 20 minutes and 2 hours, compared with control group Sham MT application and on a third group taking only blood sampling. Whole blood cultures were activated with lipopolysaccharide for 24 h, and specific immunoassays determined the production of TNF-α, IL-1β, and substance P. Substance P remained unchanged in all three groups. In contrast, the cytokines decreased only in the treated group.

In a recent study [55], the severity of symptomatology in patients with low back pain was found parallel to IL-6 and not IL-1β, IL-8, IL-10, TNF-a values. Considering this, a six-session on an 8-week HVLA treatment protocol was implemented with blood sampling before the first session and a second one in the twelfth week. Results showed a decrease of TNFα in the second measurement, without the expected changes in IL-6 and IL-1β, which had a large baseline variation. The researchers interpreted the effect through the mechanism of stimulation of extracellular matrix proteases stimulated by cytokines. These findings can not be compared with our study because TNFα and IL-6 were not included in our measurements. According to IL-1b, in the work of Licciardone and co-workers, baseline values had significant variation statistically. In our study, a statistically significant decrease in IL-1β levels was found with an increased number of sessions (nine) at three weeks duration with the final measurement immediately after the last session. Licciardone implemented a 6-session program in 8 weeks, making their second measurement at 12 weeks,

that is, four weeks after the end of their intervention. Assuming the correlation is dose-dependent, a more significant number of sessions would probably lead to a larger difference.

Moreover, the kit was a multivariate analysis, compared to ours, which was a one-dimensional IL-1b kit. Our study incorporated one cytokine versus four of Licciardone, thereby statistically limiting the type I error inflation that would probably result from the increased number of paired comparisons. Also, the comparison of samples is difficult as they are relatively heterogeneous groups of the back and cervical pain patients.

Study limitations

The study conducted in a private physiotherapy clinic covering a specific demographic area, thereby limiting external validity. The sample size does not allow for the detailed statistical application of parametric statistical tests with increased power, thus improving results interpretation, mainly interactions between factors. Paraspinal muscle strength was not measured because no treatment program was implemented. So, no correlation can be assumed with pain and disability improvements. Our goal was to examine a single MT effect, besides of multifactorial treatment plan. However, this study is one of the few research efforts elucidating the mechanism of MT effect on CNP patients at a cellular level, measuring peripheral blood biomarkers.

CONCLUSION

Implementation of manual therapy techniques, in the form of HVLA/LVLA, acutely improves the clinical status of patients with chronic neck pain, thereby enhancing therapeutic guidelines of MT application. Reduction in IL-1β concentration indicates o potential mechanism of action interpreting therapeutic effects. Future research needs to in-depth examine cellular signal pathways under a comprehensive research design, with larger sample size and on different subgroups of patients with chronic neck pain (e.g., non mechanical). Triggered by this, integrated therapeutic regimens that will combine manual therapy with appropriate drug prescription needs to be extensively examined.

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