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Working Paper *in* Journal of Spinal Disorders & Techniques · December 2014

DOI: 10.1097/BSD.0000000000000231 · Source: PubMed

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Changes in Cytokines, Sensory Tests, and Self-reported Pain Levels After Manual Treatment of Low Back Pain

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Study Design: Unbalanced 3-factor design with repeated measures on 1 factor.

Objective: To determine the effect of manual treatment (MT) on cytokine and pain sensations in those with and without low back pain (LBP).

Summary of Background Data: Evidence suggests that MT reduces LBP but by unknown mechanisms. Certain cytokines have been elevated in patients with LBP and may be affected by MT.

Methods: Participants aged 20–60 years with chronic LBP or without LBP were recruited and randomly assigned to MT, sham ultrasound treatment, or no treatment groups. Venous blood samples were collected and pain levels assessed at baseline, 1 hour later, and 24 hours later. Blood was analyzed for interleukin (IL)-1β, IL-6, tumor necrosis factor-α, and C-reactive protein. Pain levels were measured by pressure pain threshold (PPT), mechanical detection threshold (MDT), dynamic mechanical allodynia, and self-report.

Results: Forty (30 women, age 36 ± 11 y) participants completed the study, 33 with LBP (13 MT, 13 sham ultrasound treatment, and 7 no treatment) and 7 without LBP. Participants with or without LBP could not be differentiated on the basis of serum cytokine levels, PPT, or MDT ($P \geq 0.08$). There were no significant differences between the groups at 1 hour or 24 hours on serum cytokines, PPT, or MDT ($P \geq 0.07$). There was a significant decrease from baseline in IL-6 for the no treatment (LBP) group ($P = 0.04$), in C-reactive protein for the sham ultrasound treatment group ($P = 0.03$), in MDT for all 3 LBP groups ($P \leq 0.02$), and in self-reported pain for the MT and sham ultrasound treatment groups ($P = 0.03$ and 0.01).

Conclusions: Self-reported pain was reduced with MT and sham ultrasound treatment 24 hours after treatment, but inflammatory markers within venous circulation and quantitative sensory tests were unable to differentiate between study groups. Therefore, we were unable to characterize mechanisms underlying chronic LBP.

Key Words: low back pain, inflammatory markers, quantitative sensory tests, manual treatment

(*Clin Spine Surg* 2017;30:E690–E701)

Low back pain (LBP) is a common cause of disability for individuals under the age of 45, and as much as 1% of the US population is chronically disabled by it.[1] Several reviews have shown that certain forms of manual treatment (MT) can be as effective as or more effective than placebo, usual care by a general practitioner, bed rest, analgesics, or massage in the treatment of chronic LBP.[2–6] Recent evidence suggests that 1 form of MT, osteopathic manipulative treatment, has up to a medium effect size for relieving acute and chronic LBP.[7] This evidence is the foundation for the American Osteopathic Association's clinical practice guideline on the use of osteopathic MT in the management of LBP.[8] However, little is known about the underlying physiological mechanisms of MT.

There are 2 broad categories that define mechanisms for pain: nociceptive and neuropathic. Nociceptive pain is produced in normal tissue secondary to tissue damage, potentially damaging physical strain or chemical irritation, and the subsequent inflammation.[9] When nerve tissues sustain injury or functionally become altered because of repeated stimulation, which may be the circumstances underlying chronic LBP, nociceptors may become sensitized and increase their rate of firing to stimuli that had previously not caused nerve activation, a phenomenon known as allodynia. Allodynia is an indication of neuropathic pain and central sensitization.[10,11]

Although some evidence indicates that MT is useful in reducing LBP, previous MT studies have not discriminated whether the treated LBP had nociceptive and/or neuropathic origins. To investigate the impact of MT on the different neurophysiological mechanisms of LBP, current methods for evaluating nociceptive and neuropathic pain in relation to MT need to be studied. Nociceptive pain can be assessed by measuring circulating concentrations of proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and C-reactive protein (CRP).[12,13] Each of these cytokines has been implicated in LBP in humans or animal models.[14–16] Further, IL-1β,

Received for publication August 21, 2014; accepted November 17, 2014.
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Funded by an American Osteopathic Association grant, #06-04-550. The authors declare no conflict of interest.
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IL-6, and TNF-α are produced locally at sites of inflammation by T cells, macrophages, and other inflammatory leukocytes, whereas CRP is released from the liver in response to IL-6 stimulation. Cells other than leukocytes (eg, fibroblasts) can also produce IL-1β, IL-6, and TNF-α in response to mechanical stretch or other stimuli.[17–19] If chronic LBP is associated with inflammation, then effective MT designed to relieve tissue tightness and thus strain on nerves and microvasculature, secondarily improving local circulation, may reduce pain by reducing the concentration of these cytokines in the affected zone.

Quantitative sensory tests (QSTs) have been used experimentally and clinically to evaluate neuropathic pain. There are 7 QSTs measuring 13 parameters of sensation, assessing and quantifying the perception of temperature, touch, pain, pressure, and vibration.[20–22] Historically, the duration and lack of standardization in performing these tests and their lack of influence on treatment decision making has limited their clinical use. Recent standardization for QSTs has improved the reliability and clinical usefulness of these tests.[21–24] Also, several manipulation studies have used QSTs in their study designs and showed an increase in pressure pain thresholds (PPT) after manipulation but no changes to thermal pain stimuli.[25,26] Consequently, in the current study, thermal thresholds (pain and detection) were not evaluated.

A recently published study on adults with nonspecific, chronic LBP failed to demonstrate changes from baseline levels of inflammatory cytokines after 6 osteopathic MTs over a 12-week period.[27] The follow-up cytokine testing occurred 4 weeks after the last treatment to evaluate a longer-term effect of MT on pain and cytokine levels. As the half-life of cytokines commonly investigated in this field ranges from 15 minutes to 24 hours,[28–30] the immediate impact of MT on these markers is currently unknown. Therefore, in the current study, we evaluated the body's response to MT over a 24-hour period.

The current study was designed as a pilot investigation to evaluate several questions: do people with chronic LBP have higher levels of IL-1β, IL-6, TNF-α, and CRP than people without LBP; do people with chronic LBP have signs of neuropathic pain; does MT produce immediate reduction in pain in people with chronic LBP and is that reduction in pain correlated with changes in circulatory markers of inflammation over a 24-hour period; and lastly, does MT cause changes in abnormal baseline QSTs. We hypothesized that chronic LBP would have greater nociceptive versus neuropathic pain characteristics and that MT would reduce pain and circulatory markers of inflammation over a 24-hour period.

MATERIALS AND METHODS

The experimental design for the current study was an unbalanced 3-factor design with repeated measures on 1 factor (Fig. 1). From January to November 2007, participants with either chronic LBP or without LBP were recruited at a ratio of 5:1. LBP participants were then assigned to 1 of 3 treatment groups—MT, sham ultrasound treatment, or no treatment—using unbalanced blocked randomization with a block size of 5. Within each block of 5, 2 participants were assigned to the MT group, 2 participants to the sham ultrasound treatment group, and 1 participant to the no treatment group. We used 3 types of control participants: a placebo control group (LBP with sham ultrasound treatment), a no treatment control group (LBP with no treatment), and a negative control group (no LBP). Outcomes were measured at baseline, 1 hour later, and 24 hours later. For each data collection time, participants were evaluated for inflammatory cytokines, QSTs, and current perceived pain using a 0–10 point numerical pain scale. The local institutional review board approved the study.

Participants

Men and women aged 20–60 years were recruited from an 8-county underserved region in northeastern Missouri by e-mail at 2 local universities and by advertisement in the local newspaper and public flyers. For the LBP groups, participants had a self-reported history of nonradicular LBP for a minimum of 5 days a week for at least 6 weeks, and their average pain level was at least a 4 on the numerical pain scale. For the no LBP group, participants had no self-reported history of pain in the past 6 weeks.

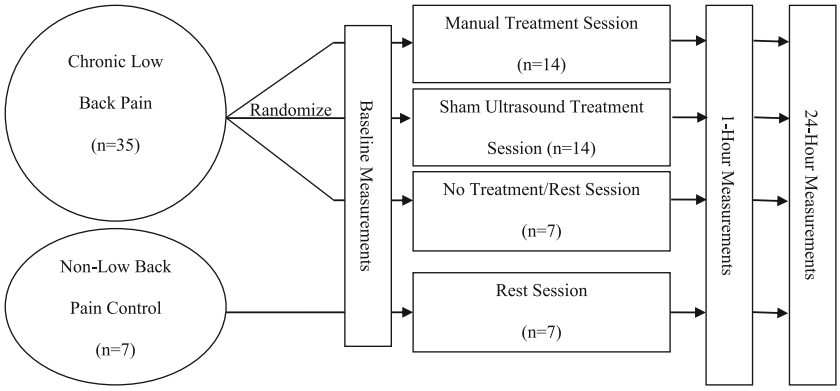

FIGURE 1. Experimental design flowchart.

Potential participants were excluded from the study if they had one of the following: lumbar or low thoracic spinal fractures; known congenital vertebral abnormalities of the lumbar spine, such as spina bifida; moderate to severe scoliosis; lumbar or sacral radiculopathy; spinal surgery in the lumbar or low thoracic region; a history of persistent numbness or weakness in a leg or the legs; a history of claudication, cauda equina syndrome, or cancer (excluding nonmalignant skin cancer); current infections or inflammatory processes, such as ankylosing spondylitis, spinal osteomyelitis, or urinary tract infection; an autoimmune disease; the inability to maintain the positions necessary for treatment; MT of the spine within 4 weeks of the initial musculoskeletal examination; use of corticosteroids during the past 3 months; an unwillingness to stop taking nonsteroidal anti-inflammatory drugs or aspirin for the 24 hours before the study and until after the follow-up examination; or a body mass index >30.

All participants signed an informed consent form before participating in the study.

Procedures

First, participants completed a general demographic data form, an LBP history form, an 11-point numeric rating scale for current stress level (0 = no stress, 10 = extreme stress), and a self-reported scale of perceived pain with an 11-point numeric rating scale with faces (0 = no pain, 10 = severe pain). Participants then had a blood sample drawn by venipuncture for determination of baseline cytokine concentrations. Next, all participants were given a structural examination by the same board-certified neuromusculoskeletal specialist (B.F.D.). After examination, 4 standardized locations (the spinous processes of L2 and L4 and just inferior to the posterior superior iliac spines) were marked by the specialist with permanent marker for PPT testing. Up to 2 nonstandardized locations (participant-specific structures having more pain on palpation than the standardized locations) could also be marked to better localize their symptoms for PPT testing. One of 2 trained technicians (C.F., C.T.A.), blinded to the participant's treatment group and palpatory examination findings, performed the baseline QSTs.

After all baseline data were collected, participants randomized to the MT group or the sham ultrasound treatment group received their intervention over a 20-minute period. The participants then rested for 40 minutes in a comfortable position. Those in the no treatment and no LBP groups primarily remained seated for the hour after baseline data collection. For the 1-hour measurements, LBP participants completed a new self-reported pain scale and all participants had blood drawn to determine cytokine concentrations. Another structural examination and repetition of the QSTs followed.

Twenty-four hours later, participants returned for final measurements. After resting 10 minutes, LBP participants completed a self-reported pain scale. The structural examination and QSTs were repeated for all participants, and blood was drawn to determine cytokine concentrations.

Treatment Protocols

The MT protocol was 90% standardized and followed current standards of osteopathic techniques.[31] Treatment began with the participant in the prone position. The sacrum was treated using indirect and springing techniques. The sacroiliac joints were gapped and soft tissue kneading was performed to the gluteal muscles and lumbosacral erector spinae. Indirect and gentle direct positional release techniques were then performed to improve lumbar segmental motion. Muscle energy was used to stretch hypertonic hip flexors. With the participant in the lateral recumbent position, a lumbar roll was performed within the participant's tolerance for such positioning and forces. Participants then turned onto their backs. Pubic decompression technique was performed if pubic dysfunction was noted. Muscle energy technique was used to stretch hypertonic hip and lumbar musculature. An articulatory sacroiliac joint technique was performed to complete the treatment. If some component of the participant's somatic dysfunction did not adequately respond, the treating specialist (B.F.D.) would add or modify a technique to optimize the treatment outcome for each participant. This treatment lasted approximately 20 minutes.

Participants who received sham ultrasound treatment were in the prone position. An unpowered ultrasound head was moved gently across their back for 20 minutes in conjunction with pleasant conversation.

Inflammatory Cytokine Concentrations

Blood was collected by venipuncture from each participant into separate 10 mL red-top Vacutainers (Becton Dickinson, Franklin Lakes, NJ) at baseline, 1 hour later, and 24 hours later. Blood was allowed to clot for 2 hours at 4°C before the serum was separated by centrifugation at 400*g* for 5 minutes. The sera were stored at −80°C in single-use aliquots until analyzed. Concentrations of proinflammatory cytokines were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Table 1) according to the manufacturers' instructions. In some serum samples, concentrations of IL-1β, IL-6, and TNF-α were below the limits of detection of standard sensitivity kits; in these instances, the sera were further evaluated using ultrasensitive kits. Cytokine concentrations below the limits of detection for all measurements were given a value of 0. All serum samples were assayed in duplicate. For consistency, we measured the concentration of 1 cytokine for all 3 blood draws from a participant on the same plate. Absorbances were read at 450 nm in a Labsystems Multiskan MCC/340 ELISA reader (Fisher Scientific, St Louis, MO). Linear regression curves were constructed for each plate using duplicate samples of 5 known concentrations and were used to derive the inflammatory marker concentrations in each serum sample. Intra-assay coefficients of variation (CV) were calculated for the derived cytokine concentrations from each plate and then pooled over all the plates.[32] Serum samples that yielded cytokine concentrations that differed by >2-fold between duplicate replications of the same

TABLE 1. Enzyme-linked Immunosorbent Assay Kit Sensitivities

Cytokine	Kit*	Cat. No.	Sensitivity
IL-1β	Standard sensitivity	KHC0012	1 pg/mL
	Ultrasensitive	KHC0013	0.06 pg/mL
IL-6	Standard sensitivity	KHC0062	2 pg/mL
	Ultrasensitive	KHC0063	0.104 pg/mL
TNF-α	Standard sensitivity	KHC3012	1.7 pg/mL
	Ultrasensitive	KHC3013	0.09 pg/mL
CRP	Standard sensitivity	CYT298	0.0002 μg/mL

*IL-1β, IL-6, and TNF-α were measured with kits purchased from Invitrogen (Carlsbad, CA). CRP was measured with a kit purchased from Chemicon International (Temecula, CA).

Cat. No. indicates catalog number; CRP, C-reactive protein; IL, interleukin; TNF, tumor necrosis factor.

sample assayed on the same plate were retested when there was sufficient serum and were excluded from CV calculations and statistical analysis when there was insufficient serum for retesting.[33] Statistical analysis was conducted on the mean of the duplicate cytokine concentrations for each serum sample.

QSTs

For QSTs, a technician assessed PPT using pressure algometry, mechanical detection threshold (MDT) using Von Frey monofilaments, and dynamic mechanical allodynia (DMA) using a cotton swab and a standardized brush. For PPT, the technician used a pressure gauge device (Wagner FDIX, Greenwich, CT) to determine the lowest pressure that caused pain in the 4–6 locations marked by the neuromusculoskeletal specialist. To standardize the locations where MDT and DMA were performed, a flexible, 5-column, 2-row grid (evenly distributed in an area about 8.5×5 inches) was placed on the participant's back to identify 10 testing sites, which were marked with a permanent marker. The grid was placed with the middle column centered along the spinous processes of L4 and S1. The adjacent columns approximately overlaid the transverse processes of the same vertebrae or the sacroiliac joints, and the lateral columns were along the lateral border of the erector spinae mass. For MDT, calibrated Von Frey monofilaments (North Coast Medical, Morgan Hill, CA) were used to determine the force at which the participant identified being touched. For DMA, a cotton swab and standardized brush were used to determine the lowest one of these non-noxious stimuli that the participant reported as painful. For the QSTs, each of the testing sites was evaluated 3 times in a random order and the mean of the 3 evaluations (arithmetic for PPT and geometric for MDT and DMA) was used in the analyses.

Statistical Analyses

The groups were compared on age, body mass index, stress level, baseline cytokine concentrations, and self-reported pain using Kruskal-Wallis tests, and multiple comparisons were completed when appropriate using the Dunn procedure. Fisher exact tests were used to compare the groups on sex and previous experience with receiving MT. Within-group comparisons were performed on the cytokine concentrations and self-reported pain using Friedman tests. Nonparametric analysis of covariance was conducted to compare the groups on the 1-hour and 24-hour cytokine concentrations and self-reported pain wherein the baseline level of the outcome variable was included as a covariate. General linear mixed models were used to test for within-group and between-group differences on PPT, MDT, and DMA to include data from all testing sites. For the between-group comparisons at 1 hour later and 24 hours later, the baseline level of the outcome variable was included in the model as a covariate. Because the distribution of the MDT data was approximately log-normal, the log-transformed data were used in the analysis of the MDT data. P values < 0.05 were considered to be statistically significant. SAS version 9.3 (SAS Institute Inc., Cary, NC) was used to conduct the statistical analyses.

RESULTS

Forty-two participants were recruited for the current study. One participant withdrew from the study after the first day, and 1 participant was excluded due to an infection. Thirty-three participants (82%) had LBP and 7 (18%) had no LBP. LBP participants were randomized into 3 treatment groups. There were 13 participants in the MT group, 13 in the sham ultrasound treatment group, and 7 in the no treatment (LBP) group. Participant demographics are summarized in Table 2. There were no significant differences between groups in sex, age, body mass index, duration of LBP, or stress level.

For many serum samples, concentrations of IL-1β and IL-6 were below the limits of detection regardless of whether standard sensitivity or ultrasensitive ELISA kits were used to measure the cytokines. Serum concentrations of IL-1β fell within the valid range in just 7 (6%) of 120 individual blood draws, yielding values ranging from 0.07 to 0.11 pg/mL. The intra-assay CV for IL-1β was 5%. Serum concentrations of IL-6 fell within the valid range in 39 (34%) of 114 samples, with concentrations ranging from 0.11 to 52.90 pg/mL; the volume of serum was inadequate from 1 participant for measuring IL-6 concentrations and was insufficient for retesting for 1 serum sample from each of 3 participants. The intra-assay CVs for IL-6 were 9% for the standard sensitivity kit and 20% for the ultrasensitive kit. Nine individual serum samples contained less TNF-α than could be reliably detected with the ELISA kits; the remaining 111 (92%) samples yielded concentrations of TNF-α between 0.16 and 22.61 pg/mL. The intra-assay CVs for TNF-α were 7% for the standard sensitivity kit and 8% for the ultrasensitive kit. CRP was detected in all 120 (100%) serum samples and ranged in concentration from 0.04 to 38.29 μg/mL. The intra-assay CV for CRP was 7%.

Comparisons of the LBP and no LBP participants at baseline are presented in Table 3. There were no significant differences between the LBP and no LBP partic-

TABLE 2. Demographics of Study Participants

Variable	All (n = 40)	MT (n = 13)	Sham Ultrasound Treatment (n = 13)	No Treatment (n = 7)	No LBP (n = 7)	P*
Sex (female) [n (%)]	29 (72)	11 (85)	9 (69)	4 (57)	5 (71)	0.55
Age (mean ± SD) (y)	36 ± 11	39 ± 13	35 ± 12	39 ± 10	32 ± 9	0.64
BMI (mean ± SD)	25.1 ± 4.3	25.1 ± 4.8	25.0 ± 4.1	26.1 ± 4.1	24.1 ± 4.3	0.73
LBP duration† (mean ± SD) (y)	9.1 ± 6.3	10.7 ± 8.2	7.4 ± 5.6	9.7 ± 2.5		0.52
Past MT [n (%)]	28 (70)	9 (69)	10 (77)	3 (43)	6 (86)	0.36
Stress [median (Q1–Q3)]	3.8 (2.0–6.0)	3.0 (1.0–5.0)	4.0 (2.0–6.0)	4.0 (3.5–6.0)	2.0 (1.0–6.0)	0.62

*Fisher exact test or Kruskal-Wallis test comparing groups.
†n = 32 participants with LBP.
BMI indicates body mass index; LBP, low back pain; MT, manual treatment; Q, quartile.

ipants at baseline on any of the cytokine data or QST results ($P \geq 0.08$). The only significant difference between LBP and no LBP participants was for self-reported pain ($P < 0.001$).

The cytokine concentration data are presented in Table 4. There were no significant differences at baseline between the groups in IL-1β ($P = 0.41$), IL-6 ($P = 0.07$), or TNF-α ($P = 0.45$). However, CRP concentrations at baseline were significantly different between the groups ($P = 0.04$), with the no treatment (LBP) group having higher concentrations than the MT group. The no treatment (LBP) group was the only group in which the median CRP concentration was above the normal range of < 1.0 μg/mL. There were no significant differences between the groups in any of the cytokines at either 1 hour or 24 hours ($P \geq 0.06$). There was no significant change within any of the groups for IL-1β ($P \geq 0.20$) or TNF-α ($P \geq 0.11$). There was a significant change over time in IL-6 for the no treatment (LBP) group ($P = 0.04$) with the concentrations at 24 hours being lower than the baseline concentrations. There was a significant change over time in CRP for the sham ultrasound treatment group ($P = 0.03$) with the baseline concentrations being higher than the other 2 times.

DMA was detected in only 4 participants. The PPT and MDT data are presented in Table 5. There were no significant differences between the groups in either PPT or MDT at baseline, 1 hour later, or 24 hours later ($P \geq 0.09$). There was no significant change within any of the groups for PPT ($P \geq 0.39$). There was a significant change over time in MDT for the MT group ($P = 0.02$) with MDT being lower at 24 hours than the other 2 times, for the sham ultrasound treatment group ($P < 0.001$) with MDT being higher at baseline than the other 2 times, and for the no treatment (LBP) group ($P = 0.005$) with MDT being higher at baseline than 24 hours later.

There was a significant change in self-reported pain in the MT and sham ultrasound treatment groups ($P = 0.03$ and 0.01) with the baseline pain levels being higher than the other 2 times (Table 6). There were no significant differences between the 3 LBP groups on self-reported pain at any of the 3 times ($P \geq 0.37$).

DISCUSSION

Cytokines are implicated in the generation of pain at peripheral and central nervous system sites. In addition, IL-1β, IL-6, and TNF-α have proalgesic effects if injected

TABLE 3. Baseline Cytokines, Quantitative Sensory Tests, and Self-reported Pain

Variable	LBP (n = 33)	No LBP (n = 7)	P*
Cytokines (median, Q1–Q3)			
IL-1β† (pg/mL)	0, 0–0 (max = 0.11)	0, 0–0 (max = 0)	0.28
IL-6† (pg/mL)	0, 0–0.46 (max = 52.90)	0, 0–0 (max = 0.18)	0.09
TNF-α (pg/mL)	3.68, 1.45–5.85	0.60, 0.37–6.51	0.23
CRP (μg/mL)	0.82, 0.40–2.48	0.65, 0.56–6.71	0.71
Quantitative sensory tests (mean ± SD)			
PPT (kg/cm^2)	3.7 ± 1.6	4.3 ± 1.0	0.53
MDT (mN)	0.9 ± 1.0	1.2 ± 0.8	0.23
Self-reported pain (median, Q1–Q3)			
Pain (11-point scale)	4, 3–6	0, 0–2	< 0.001

*Comparing between LBP and no LBP participants. For cytokines and pain, *P* value from Mann-Whitney test. For quantitative sensory testing, *P* value from general linear mixed model.
†Because of the large number of samples with values below the detection level, the maximum value (max) is provided in addition to the median and first and third quartiles (Q1 and Q3).
CRP indicates C-reactive protein; IL, interleukin; LBP, low back pain; MDT, mechanical detection threshold; PPT, pressure pain threshold; Q, quartile; TNF, tumor necrosis factor.

TABLE 4. Changes in Cytokine Levels Over a 24-Hour Period After Manual Treatment, Sham Ultrasound Treatment, or No Treatment (Median, Q1–Q3)

Cytokine	Time	MT (n = 13)	Sham Ultrasound Treatment (n = 13)	No Treatment (n = 7)	No LBP (n = 7)	P*
IL-1β† (pg/mL)	Baseline	0, 0–0 (max = 0.11)	0, 0–0 (max = 0.07)	0, 0–0 (max = 0.08)	0, 0–0 (max = 0)	0.41
	1 h	0, 0–0 (max = 0.08)	0, 0–0 (max = 0)	0, 0–0 (max = 0.07)	0, 0–0 (max = 0)	0.56
	24 h	0, 0–0 (max = 0)	0, 0–0 (max = 0)	0, 0–0 (max = 0)	0, 0–0 (max = 0)	> 0.99
	P‡	0.20	0.37	0.37	NA	
IL-6† (pg/mL)	Baseline	0, 0–0.14 (max = 0.51)	0.18, 0–0.46 (max = 52.90)	1.75, 0–9.85 (max = 24.20)	0, 0–0 (max = 0.18)	0.07
	1 h	0, 0–0.06 (max = 0.22)	0, 0–5.63 (max = 42.32)	1.93, 0–8.25 (max = 19.30)	0, 0–0 (max = 0.21)	0.56
	24 h	0, 0–0.06 (max = 0.36)	0, 0–0.54 (max = 44.28)	0, 0–0.98 (max = 13.15)	0, 0–0 (max = 0.40)	0.06
	P‡	0.09	0.25	0.04	0.37	
				Baseline > 24 h		
TNF-α (pg/mL)	Baseline	3.45, 0.66–4.79	4.05, 1.45–6.54	3.12, 0.91–5.85	0.60, 0.37–6.51	0.45
	1 h	3.56, 1.15–4.90	2.90, 1.56–4.24	3.68, 0.78–5.79	0.62, 0.44–5.81	0.26
	24 h	4.36, 2.05–4.79	2.78, 1.91–4.89	3.34, 0–5.81	0.66, 0.51–6.18	0.63
	P‡	0.34	0.11	0.96	0.49	
CRP (μg/mL)	Baseline	0.43, 0.23–0.85	0.82, 0.42–1.72	3.47, 1.08–5.38	0.65, 0.56–6.71	0.04
						MT < no treatment
	1 h	0.57, 0.24–0.84	0.64, 0.43–1.60	3.94, 0.88–6.09	1.00, 0.55–7.57	0.59
	24 h	0.43, 0.29–0.94	0.51, 0.30–1.60	4.77, 0.74–6.79	0.97, 0.60–7.88	0.19
	P‡	0.58	0.03	0.56	0.56	
			Baseline > 1 h and 24 h			

*For baseline, Kruskal-Wallis test comparing between groups. For 1 hour and 24 hours, nonparametric analysis of covariance comparing between groups, covarying on the baseline.
†Because of the large number of samples with values below the detection level, the maximum value (max) is provided in addition to the median and first and third quartiles (Q1 and Q3).
‡Friedman test comparing within group.
CRP indicates C-reactive protein; IL, interleukin; LBP, low back pain; MT, manual treatment; Q, quartile; TNF, tumor necrosis factor.

subcutaneously into normal tissue.[13] Administration of exogenous IL-6 or TNF-α to normal dorsal root ganglia causes pain and enhances preexisting hypersensitivity caused by mechanical compression of the dorsal root ganglia.[34,35] Studies conducted in rats have shown that mechanical and chemical injuries to lumbar roots in the spinal cord result in enhanced expression of IL-1β, IL-6, and TNF-α.[35–38] Pain can be dramatically reduced by administering monoclonal antibodies or soluble receptors that act as competitive antagonists for IL-1β, IL-6, TNF-α, or their receptors.[39–45] Herniated disks spontaneously produce pain-inducing nitric oxide, IL-8, cyclooxygenase-2, and phospholipase A_2 in addition to TNF-α, IL-1, and IL-6.[46] Although there is evidence that proinflammatory cytokines may act directly on nociceptors,[47] they contribute to pain hypersensitivity mainly by potentiating the inflammatory response and increasing production of proalgesic agents, such as prostaglandins, nerve growth factor, bradykinin, and extracellular protons.[48]

Serum CRP is used as a general index of inflammation caused by trauma, infection, surgery, tumors, and myocardial infarction.[49] In normal serum, CRP is present at concentrations < 1.0 mg/L.[50] Using standard sensitivity tests, Laird et al[51] found a positive correlation between pain and CRP concentrations in a cohort of patients with gastrointestinal, lung, or pancreatic cancer. Le Gars et al[52] showed that CRP concentrations were significantly higher in patients with disk-related lumbosciatic syndrome than in normal age-matched and sex-matched controls, a result consistent with a systemic inflammatory response to the local nerve root impingement. Small increases in high-sensitivity CRP have been linked to the development of pain conditions, such as rheumatoid arthritis and fibromyalgia.[53,54] A study conducted in 99 pairs of female twins demonstrated that clinically important concentrations of high-sensitivity CRP (> 3.0 μg/mL) are associated with greater cold pain sensitivity after controlling for age, body mass index, pain status, and time until pain threshold is reached.[12]

In the current study, the participants in each group demonstrated no statistical differences in sex, age, body mass index, duration of LBP, stress, or use of MT for LBP. Despite the tightly controlled population we studied and the abovementioned evidence that LBP is associated with elevations in the inflammatory markers we evaluated, we were unable to show meaningful differences in serum concentrations of IL-1β, IL-6, TNF-α, and CRP between LBP and no LBP participants. As a result, these serum-derived cytokines were not adequate markers for pain in our cohort. Therefore, we were unable to determine whether IL-1β, IL-6, TNF-α, and CRP concentrations were altered by standardized MT for LBP.

Only 6% of the samples in our study had serum concentrations of IL-1β that fell within the valid range determined by the standards. This percentage is lower than that obtained by Antonelli et al,[55] who detected IL-1β in 30 of 43 (70%) normal control serum samples. The median IL-1β concentrations obtained by Antonelli et al[55] and by Laban-Guceva et al[56] in normal control sera were 0.7 and 0.24 pg/mL, respectively. The highest

TABLE 5. Changes in Quantitative Sensory Tests Over a 24-Hour Period After Manual Treatment, Sham Ultrasound Treatment, or No Treatment (Mean ± SD)

Variable	Time	MT (n = 13)	Sham Ultrasound Treatment (n = 13)	No Treatment (n = 7)	No LBP (n = 7)	P*
PPT (kg/cm^2)	Baseline	3.5 ± 1.7	3.6 ± 1.5	4.3 ± 1.4	4.3 ± 1.0	0.75
	1 h	3.5 ± 1.8	3.5 ± 1.6	4.6 ± 1.4	4.5 ± 1.1	0.29
	24 h	3.6 ± 1.6	3.5 ± 1.7	4.4 ± 1.5	4.5 ± 1.0	0.39
	P†	0.67	0.71	0.48	0.39	
MDT‡ (mN)	Baseline	0.7 ± 0.6	1.2 ± 1.4	0.7 ± 0.7	1.2 ± 0.8	0.09
	1 h	0.7 ± 0.7	0.9 ± 1.1	0.6 ± 0.6	1.0 ± 0.6	0.54
	24 h	0.6 ± 0.6	0.8 ± 0.9	0.5 ± 0.4	1.0 ± 0.7	0.42
	P‡	0.02	< 0.001	0.005	0.06	
		Baseline and 1 h > 24 h	Baseline > 1 h and 24 h	Baseline > 24 h		

*For baseline, general linear mixed model comparing between groups. For 1 hour and 24 hours, general linear mixed model comparing between groups, covarying on the baseline.
†Friedman test comparing within group.
‡Log-transformed data were analyzed.
LBP indicates low back pain; MDT, mechanical detection threshold; MT, manual treatment; PPT, pressure pain threshold.

serum concentration of IL-1β detected in any of our study participants was 0.11 pg/mL.

For IL-6, 34% of the samples from our study participants had concentrations that fell within the valid range determined by the standards. This percentage contrasts with results from the study by Antonelli et al,[55] who detected IL-6 in 100% of normal controls. Various investigators have reported median IL-6 concentrations of 0.8,[55] 1.72,[56] and 2.9 pg/mL.[57] In the current study, we were unable to detect any concentration of IL-6 in the no LBP group, and in the LBP group our median was 0 pg/mL with first through third quartiles from 0 to 0.46 pg/mL. One would expect higher serum concentrations of IL-6 in all participants with LBP versus those without LBP, although the differences between our groups do not support such a conclusion. Although a statistically significant decrease in IL-6 concentrations was seen in the no treatment (LBP) group over the 24-hour period in the current study, interpretation of these findings is problematic as a large number of samples (66%) had concentrations below the level of detection.

For TNF-α, 92% of the samples in our study provided values that fell within the valid range determined by the standards, with a median concentration of 3.68 pg/mL. This percentage of findings is similar to that observed by Antonelli et al,[55] who detected TNF-α in 38 of 43 (88%) normal controls, with a median concentration of 1.1 pg/mL. In the current study, the median concentration of TNF-α in participants with LBP was >6-fold higher than in participants without LBP. However, no statistically significant difference was seen between these 2 groups potentially because of the small sample size. Although a larger sample size is necessary to determine whether this marker differentiates between those with and without LBP, none of our interventions influenced TNF-α concentrations over the 24-hour period.

In previous back pain studies in which cytokines were measured in human serum, results have been variable. Kraychete et al[14] demonstrated that circulating concentrations of IL-6 and TNF-α, but not IL-1β, were higher in patients with herniated disks than in healthy controls. In contrast, Yang et al[58] showed that circulating IL-1β increased significantly over time in subjects who participated in a study to determine the acute biochemical responses to physical work stressing the low back. For the participants in our study, the etiology of the LBP was unknown. It has been previously estimated that most cases of LBP (85%) arise from nonspecific biomechanical origins.[2,59–62] Therefore, nonspecific biomechanical origins were likely the etiology of the LBP in a majority of our participants, limiting our ability to compare our results with those of Kraychete et al.[14] Although the current study's population was likely more comparable to the population in the study by Yang et al,[58] our outcomes in cytokine concentrations were also inconsistent with those reported in that study.

TABLE 6. Changes in Pain in Low Back Pain Participants Over a 24-Hour Period After Manual Treatment, Sham Ultrasound Treatment, or No Treatment (Median, Q1–Q3)

Variable	Time	MT (n = 13)	Sham Ultrasound Treatment (n = 13)	No Treatment (n = 7)	P*
Self-reported pain (11-point scale)	Baseline	4.5, 3–7	4, 3–4	4, 2–6	0.53
	1 h	3, 0.5–5	3, 1–3	3.5, 2–6	0.37
	24 h	3, 2–5	2.5, 2–3	3.5, 2–5	0.51
	P†	0.03	0.01	0.64	
		Baseline > 1 h and 24 h	Baseline > 1 h and 24 h		

*For baseline, Kruskal-Wallis test comparing between groups. For 1 hour and 24 hours, nonparametric analysis of covariance comparing between groups, covarying on the baseline.
†Friedman test comparing within group.
MT indicates manual treatment; Q, quartile.

CRP is a molecule produced by the liver when there is inflammation occurring somewhere within the body. It is a nonspecific yet sensitive marker of inflammation. In the current study, the expected elevation in CRP concentrations in the LBP group was not seen. Although the statistically significant decrease in the CRP concentrations 1 hour and 24 hours after sham ultrasound treatment may indicate a placebo response, these readings were generally within the normal range for this group and so have unknown clinical relevance. As CRP is easily influenced by inflammation anywhere in the body, future use of CRP for the evaluation of LBP should be cautiously considered. More stringent exclusion criteria should be considered to reduce the potential of other sources of inflammation that may skew assessment of the inflammation produced at the site of the LBP.

In the current study, the study group, intervention, and outcome measures are similar to those reported in a study by Licciardone et al.[27] In that study,[27] adult participants had chronic LBP, and serum samples were taken to evaluate for IL-1β, IL-6, IL-8, IL-10, and TNF-α. Overall, the type of treatment techniques performed and the duration of treatment were similar between our study and the Licciardone study. Whereas we evaluated the impact of osteopathic MT on LBP and serum markers over a 24-hour period, Licciardone et al[27] evaluated the longer-term impact of osteopathic MT over a 12-week period. During those 12 weeks, the participants received osteopathic MT or sham ultrasound 6 times, and blood was drawn at baseline and 4 weeks after the last treatment (week 12). Although differences in the reporting of data did not allow for direct comparison between these 2 studies, a significant number of samples in the Licciardone study[27] had very low and potentially no detectable levels of IL-1β and IL-6 (in approximately 65% and 58% of the samples, respectively). In our study, a higher percentage of sera contained IL-1β and IL-6 at concentrations below the limit of detection (94% and 66%, respectively). In addition, the results for IL-8 and IL-10 in the study by Licciardone et al[27] were very low and potentially undetectable for approximately 63% and 45% of samples, respectively. This combined evidence suggests that the use of serum for assessing these cytokines may be inadequate to distinguish those who have LBP from those who do not, particularly if part of the mechanism for the effect of MT in this population alters the concentrations of these cytokines.

In both our study and the study by Licciardone et al,[27] TNF-α could be reliably and consistently quantified. The median (interquartile range) TNF-α concentration was 5.7 (3.6) pg/mL in the Licciardone study,[27] and in our study the median concentration was 3.68 pg/mL (first through third quartiles, 1.45–5.85 pg/mL). Although our study failed to demonstrate changes in serum concentrations of TNF-α 24 hours after a single MT or sham ultrasound treatment, the study by Licciardone et al[27] found a significant decrease in serum concentrations of TNF-α 12 weeks after patients received a series of 6 osteopathic MTs compared with those who received sham MT. These different findings for the influence of MT on TNF-α concentrations in the serum between our study and the Licciardone et al[27] study could have resulted from differences in dosing, the follow-up period, or unique aspects of the MT protocols.

Changes in other biomarkers after MT have been studied in chronic LBP populations. In 1 study,[63] a cohort of LBP participants was compared with a sex-matched and age-matched control group. In that study, both groups received MT applied pragmatically to each participant's specific areas of dysfunction. Serum β-endorphin increased 30 minutes after MT in both groups and persisted at 24 hours in chronic LBP participants. Plasma 5-hydroxyindoleacetic acid, a serotonin derivative, was reduced at 30 minutes after MT, and at 24 hours after MT serotonin was reduced significantly from baseline concentrations in the chronic LBP group. Anandamide, an endocanabinoid, was reduced at both 30 minutes and 24 hours after MT in the nonchronic LBP group, but did not change in the chronic LBP group. An increase in palmitoylethanolamide, an endogenous analog to anandamide, in chronic LBP participants 30 minutes after MT was 6 times the increase seen in the nonchronic LBP group, but concentrations approached baseline at 24 hours after MT. These marker changes correlated with reductions in pain associated with MT. However, this study should be repeated with larger sample sizes to increase the validity of these findings.

Several factors may explain why the current study failed to support our hypotheses. If the cytokines were produced locally at the site of pain as indicated by other reports,[46,64] they could have been diluted in the systemic circulation to the point of being undetectable, or they may have been rendered unavailable for binding to antibodies in the ELISA by interaction with soluble receptors or other serum components that bind to cytokines, that is, serum albumin or α_2-macroglobulin.[65] Alternatively, the cytokines may have degraded at a rate faster than expected. A related factor may be the timing for blood sampling. There were no previously published data on alterations in circulating cytokine production over a 24-hour period after osteopathic MT, so we speculated on the best time course for blood draws. The cytokines we measured vary widely in their serum half-lives: 15 minutes for IL-1β,[28] 103 minutes for IL-6,[30] 70 minutes for TNF-α,[30] and 20 hours for CRP.[29] Therefore, even if MT had an immediate inhibitory effect on cytokine production, clearance of each cytokine from the circulation would be dependent on its unique rate of decay. We reasoned that analysis of a blood draw 1 hour after baseline, and 30 minutes after completion of the interventions, would detect rapid changes in cytokines with short half-lives (IL-1β, IL-6, and TNF-α) and that a blood draw 24 hours after baseline would detect changes in the long-lived CRP. Our protocol was based on the findings of Li et al,[66] who showed that after renal artery stenting IL-6 increased within the first hour, remained elevated at 6 hours, and returned to baseline at 24 hours, whereas CRP remained at baseline 1 hour after stenting and peaked at 24 hours.

Even though different timing for sampling could be considered, we do not think that such changes in the methodology would have changed the outcomes of the current study. In addition, variations in serum collection and storage conditions, and natural alterations in cytokine levels among participants likely contributed to the disparities observed between our findings and those previously published.

Another potential reason for the current study's outcomes is that the double-antibody capture ELISA format we used may simply have been too insensitive to detect the levels of cytokines present in our participants' serum samples. We chose to measure cytokines in the peripheral circulation because this method was less invasive than other previously published in vivo sampling techniques, such as saline lavage of the lumbar disk epidural space[67] or collection of intervertebral disk tissue for immunohistochemical or gene array analysis.[68] Newer antibody-based technologies, such as multiplex ELISA,[69] are reported to have better sensitivity than traditional ELISA but were unavailable to us at the time of serum collection and analysis. The ultrasensitive IL-6 ELISA in the current study had a CV (20%) much higher than the 10% intra-assay variation considered acceptable by many investigators,[32] and this variability likely contributed to the difficulty in distinguishing between groups on the basis of IL-6 cytokine concentrations.

As the participants had chronic LBP, an alternative consideration for the current study's outcomes is that the chemical changes may be occurring on a central versus peripheral level and are thus isolated from the circulatory system. This possible explanation is consistent with the idea that chronicity of pain can cause central sensitization and neuropathic pain.[70–72] Because previous literature indicates that QSTs may differentiate between nociceptive and neuropathic origins of back pain,[24,73–75] we used 3 QSTs—PPT, MDT, and DMA—to evaluate for central sensitization and neuropathic pain. PPTs evaluate A-delta and C fibers, whereas MDT and DMA assess A-beta fibers. To ensure rigor in the current study by minimizing technician-induced variability, only 2 trained technicians performed the QSTs, and each participant had only 1 technician performing the QSTs over the 3 measurement times. Further, a permanent marker was used to label certain locations for testing. These marks were used throughout the study to orient a standardized grid to ensure that the testing sites for all tests were consistent over time. For PPT testing, 4 common anatomic locations of pain in LBP patients were marked with ink and tested, and up to 2 other sites were labeled and tested if the additional sites better localized the participant's pain during the physical examination. Even with this combination of testing sites, we found no significant differences between the study groups, and no significant changes over the 3 measurement times.

Some studies have shown that QSTs are able to differentiate between those with spinal pain and controls.[70–72] In the current study, MDTs were not different between the LBP and no LBP groups at baseline, and only 4 participants had positive sites for DMA, indicating that the QSTs used in this study did not identify signs of central sensitization. However, our sample size was too small to make generalizations about the type of pain commonly seen in people with chronic LBP. In addition, MDT significantly decreased after 24 hours for all 3 LBP groups and was near statistical significance for the no LBP group. This finding suggests that the changes were not related to treatments and may have been secondary to heightened awareness because of familiarity with the testing procedure. Further, the sham ultrasound treatment group had the most significant change in MDT, suggesting that our placebo control had a therapeutic effect. This effect may be due to the persistent light pressure of the ultrasound head moving across the superficial tissues of the low back, sacral, and gluteal tissues, influencing the nerve endings that were being stimulated by the MDT procedures. A recently published meta-analysis showed a weak relationship between pain and pain thresholds,[76] so additional research is needed to clarify the cause for this variability.

Other studies[77,78] found trends for changes in PPT after MT, but there was no statistically significant change in this parameter in the current study. In a study by Imamura et al,[77] the authors found that PPTs at the L2–L3 supraspinous ligament were significantly lower in 20 chronic LBP patients compared with an equal number of non-LBP patients. In our study, PPT was tested on the supraspinous ligament at L4 and no changes were identified over the 24-hour period.

To avoid concerns in future studies that inflammatory markers may be too dilute in peripheral blood for reliable detection, researchers should consider utilizing techniques capable of evaluating interstitial fluid in the tissue of interest. In contrast, a study by Scuderi et al[67] indicated that biological samples collected directly from the site of pain are not necessarily superior to serum for the detection of inflammatory cytokines. In that study,[67] the authors failed to detect any of 25 cytokines in 50 epidural space lavage samples taken from patients with acute radiculopathy secondary to a symptomatic herniated lumbar intervertebral disk or spinal stenosis. Further, challenges with collection of interstitial fluid include the accurate localization of the site of pain and the likelihood that invasive techniques would actually alter the local chemical milieu.

A limitation of the current study was noted by the examining clinician. At times, there was a discrepancy between participant-reported pain and pain identified during the physical examination. Several participants had a self-reported pain that was higher than that elicited during the examination. At times, no pain, tight muscles, or reactive tissue was identified in participants reporting pain at a 3 or higher. This discrepancy occurred in approximately 10% of the LBP participants, and, although the sample sizes in this study were small, it is unlikely that this overreporting of pain significantly influenced outcomes. Because previous studies suggest that sensory hypersensitivity is more common in those with higher

reported levels of pain,[79–82] the chronic LBP participants in the current study may have had levels of pain that were too low to produce positive QSTs. Yet a recent meta-analysis calls this association into question.[76]

Self-reported pain was able to differentiate between those with LBP and those without LBP. The MT and sham ultrasound treatment groups, but not the no treatment (LBP) group, had statistically significant reductions in self-reported pain. As the sham ultrasound treatment was intended to be a placebo, our outcomes make it difficult to determine whether the therapeutic response was the result of a placebo response as intended or the result of the 20-minute light stimulation of superficial tissues. Future studies need to be designed to specifically address this issue.

CONCLUSIONS

Although LBP is a common condition, finding tests that consistently identify the condition and its underlying mechanisms remains problematic. Despite our attempts to precisely control the protocol of the current study, outcomes suggested that larger sample sizes are needed to determine whether statistically significant associations existed between cytokine levels and chronic LBP. However, if larger sample sizes are necessary to identify these associations, then the value of such tests in the clinical arena is questionable. Further, results of the current study suggested that people with LBP found pain relief from touch, although it is unclear which aspects of touch provided the therapeutic effect.

ACKNOWLEDGMENTS

The authors thank Patricia Lyons and Laura Snelling for their data collection support; Ronda Sprout, Laura Isaacson, and Sissel Mathisen for their phlebotomy services; Jamie Carroll for developing the figure for the manuscript; and Deborah Goggin, MA, for her helpful edits.

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