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Integrative Ligamentous Articular Strain Technique and Muscle Energy Technique for Knee Osteoarthritis: A Pilot Study.

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Abstract

Background: Integrative Ligamentous Articular Strain Technique (LAST) and Muscle Energy Technique (MET) for managing pain, function, and range of motion (ROM) in patients with knee osteoarthritis (KOA) were examined in a pilot study designed to assess the effect size of the intervention. **Methods:** This was a pre-and post-intervention study involving twenty-eight participants with mild to moderate KOA (Kellgren-Lawrence grades II-III). The intervention included eight sessions over 4 weeks, combining LAST (which focuses on joint and ligamentous structure) and MET (which focuses on muscle action and relaxation). VAS for pain, KOOS, and goniometric measurement of ROM were tracked. The data were interpreted using Friedman test ($\alpha = 0.05$). **Results:** Participants demonstrated significant improvements in VAS pain scores (pre 6.8 ± 1.2 vs. post 3.1 ± 1.5 , $P < 0.001$), and KOOS subscores (pain, symptoms, level of activities performed, and quality of life; all $P < 0.05$). Statistical significance was also recorded for improvements in knee flexion ROM by $15.3^\circ \pm 4.2^\circ$ ($P = 0.002$). **Conclusion:** In patients with KOA, a four-week integrative LAST and MET intervention resulted in meaningful clinically advantageous improvements for pain, function, and ROM. Despite limitations on sample size, these data suggest promise in combined manual therapy approaches for KOA, and further larger randomized trials are needed.

Keywords: *Functional improvement, knee osteoarthritis, ligamentous articular strain technique, muscle energy technique, pain, range of motion.*

Introduction

Knee osteoarthritis (KOA) is a chronic degenerative joint disease characterized by progressive cartilage degradation, subchondral bone remodeling, osteophyte formation, and synovial inflammation. It is a leading cause of pain and disability worldwide, significantly impairing mobility and quality of life, especially among older adults.[1] The disease process involves mechanical stress, ligamentous instability, muscle weakness, and inflammatory mediators that contribute to joint deterioration and functional limitations. [2,3] Ligamentous structures around the knee play a crucial role in maintaining joint stability and proper biomechanics. Damage or laxity in these ligaments can lead to abnormal joint motion, increased cartilage wear, and accelerated osteoarthritic changes.[4] Moreover, periarticular muscle dysfunction, such as weakness or tightness, further compromises joint stability and contributes to pain and disability in KOA patients.[5] Conventional management of KOA includes pharmacological treatments, physical therapy, lifestyle modifications, and surgical interventions for advanced cases. However, pharmacological therapies may have side effects, and surgery is often invasive and costly. [6] Therefore, non-pharmacological, conservative treatments such as manual therapy have gained attention for their potential to reduce pain, improve joint function, and delay disease progression. [7,8]. Manual therapy techniques, including Ligamentous Articular Strain Technique (LAST) and Muscle Energy Technique (MET), target both articular and muscular components of KOA. LAST focuses on correcting joint misalignments and ligamentous restrictions by applying gentle, precise manipulations to the joint capsule, ligaments, and surrounding soft tissues. This technique aims to restore joint biomechanics, reduce abnormal stresses, and improve fluid dynamics within the joint. [9]. MET complements LAST by addressing muscle imbalances through patient-initiated isometric contractions against therapist resistance. This technique facilitates muscle

lengthening, strengthens weak muscles, and enhances neuromuscular control, which is essential for joint stability and function. [10]. MET has been shown to improve range of motion (ROM), reduce pain, and enhance functional ability in KOA patients. [11] Several studies have demonstrated the effectiveness of manual therapy in reducing pain and improving function in KOA. For example, manual therapy interventions, including joint mobilizations and soft tissue techniques, have been associated with decreased pain scores as measured by the Visual Analog Scale (VAS) and improved functional outcomes as assessed the Knee Osteoarthritis Outcome Score (KOOS). [12,13] Manual therapy is also believed to modulate nociceptive input and promote endogenous pain inhibition, contributing to symptom relief. [14,15] Despite evidence supporting the individual benefits of LAST and MET, research on their combined application in KOA management remains limited. Integrating these techniques may provide synergistic effects by simultaneously addressing joint alignment and muscle function, potentially leading to greater improvements in pain reduction, ROM, and functional capacity.

This pilot study aims to evaluate the effect size of an integrative LAST and MET intervention on pain, joint function, and ROM in individuals with KOA. The findings will provide preliminary data to support the use of combined manual therapy techniques as an adjunctive treatment for KOA and guide future larger-scale clinical trials.

Methods

Participants

Twenty-eight participants diagnosed with knee osteoarthritis (KOA) were enrolled in this nonrandomized pre-post pilot study. Subjects were recruited from the outpatient physiotherapy department of Physio Move Clinic. The inclusion criteria were as follows:

Clinical diagnosis of KOA confirmed by a physician based on the American College of

Rheumatology criteria and radiographic evidence (Kellgren-Lawrence grade II or III). [6,1]

1. Age between 40 and 70 years old.
2. Experiencing knee pain for at least 3 months. [6]
3. Ability to understand and follow instructions in the Indonesian language.

Exclusion criteria included:

1. History of knee surgery or intra-articular injections (e.g., corticosteroids, hyaluronic acid) within the last 6 months. [16]
2. Presence of inflammatory arthritis (e.g., rheumatoid arthritis), ligament rupture, or meniscal tears confirmed by imaging. [1]
3. Neurological or musculoskeletal conditions affecting lower limb function. [17]
4. Use of analgesic or anti-inflammatory medications that could interfere with outcome measures and were not discontinued at least 48 hours before assessment. [6]
5. Participation in other physiotherapy or manual therapy interventions during the study period. [7]

All participants provided written informed consent prior to enrollment.

Study Monitoring and Compliance

Participants were carefully screened for ongoing treatments, including medications and supplements, prior to enrollment to avoid confounding effects on study outcomes. Those using treatments potentially interfering with manual therapy effects were excluded. Throughout the study, participants were regularly monitored and instructed to report any new treatments or changes to their existing therapies. Compliance with the intervention sessions was recorded, and adverse events were documented. [15]

Outcome Measures

The primary outcomes were adherence rate, adverse events, and feasibility of recruitment. Adherence was measured as the percentage of completed therapy sessions out of a maximum of 8 sessions (twice weekly for 4 weeks). Each

session began and ended with participant feedback, including questions about any adverse effects such as increased pain, discomfort, dizziness, or fatigue. A non-serious adverse event was defined as any minor discomfort that did not require termination of the session or withdrawal from the study. [18,7]. Recruitment feasibility was assessed by the number of participants who consented and enrolled each month. [19]

The secondary outcomes focused on evaluating pain, function, and mobility in individuals with knee osteoarthritis. Pain intensity was assessed using the Visual Analogue Scale (VAS), a validated instrument where participants rate their knee pain on a 0–10 scale, with 0 indicating no pain and 10 indicating worst possible pain. The VAS captures changes in pain severity pre- and post-intervention. [20] Knee function was measured using the Knee Injury and Osteoarthritis Outcome Score (KOOS), which evaluates five domains: pain, symptoms, activities of daily living, sport and recreation function, and knee-related quality of life. Each domain is scored from 0 to 100, with higher scores indicating better function and fewer symptoms. KOOS is widely used and validated for assessing functional status in KOA patients. [21] Range of motion (ROM) was quantified using a standard goniometer to measure active knee flexion and extension in degrees. Improved ROM reflects enhanced joint mobility and is an important functional outcome in KOA management. [22] Functional mobility was also assessed by the Timed Up and Go (TUG) test, which records the time taken to stand from a chair, walk 3 meters, turn, walk back, and sit down. Shorter TUG times indicate better functional mobility and balance. [23]

All secondary outcome measures were collected at baseline and immediately after the final treatment session by a blinded assessor to reduce bias. These measures collectively provided a comprehensive evaluation of the efficacy and safety of the integrative LAST and MET intervention in knee osteoarthritis

Study Protocol

Three licensed physiotherapists participated in this study. Their clinical experience ranged from 6 to 15 years and included specialized training in manual therapy techniques relevant to musculoskeletal disorders. [24] Baseline measurements and post-treatment assessments were conducted by trained assessors who received formal instruction on standardized evaluation procedures to ensure consistency and reliability of measurements. [25]

All three physiotherapists had advanced training specifically in LAST and MET, with a patient caseload primarily consisting of individuals with musculoskeletal dysfunctions, including knee osteoarthritis (KOA).[26] Patients were referred to the outpatient physiotherapy clinic for evaluation of their primary complaint of knee pain and functional limitation related to KOA. Eligibility for inclusion in the study was assessed by the assigned physiotherapist during the initial evaluation. Patients who met the inclusion criteria were invited to participate, and those who agreed completed the informed consent process approved by the institutional review board.

Following the baseline assessment, participants underwent an 8-session integrative manual therapy intervention combining LAST and MET, delivered twice weekly over four weeks. Each session lasted approximately 45 minutes. The decision to administer treatment over a four-week period was based on the following considerations:

1. Previous clinical studies have demonstrated that manual therapy interventions produce measurable improvements in pain and function within 3 to 4 weeks. [27]
2. A four-week duration balances sufficient exposure to the intervention while maintaining participant engagement and minimizing dropout [5]
3. Resource availability, including therapist time and patient scheduling, supported this treatment timeline as feasible for a pilot study.

To minimize bias and ensure the integrity of data collection, several measures were implemented:

1. Outcome instruments with proven reliability and validity (such as the Visual Analogue Scale, KOOS, and goniometric ROM) were utilized to improve consistency of measurements and reduce potential bias. [28]
2. Participants were blinded to the specific hypotheses and expected outcomes of the study to reduce response bias. [29] They were informed that the study aimed to evaluate the general effects of manual therapy on knee symptoms without disclosing anticipated results.
3. Self-reported outcomes were collected at multiple time points (baseline and post-intervention) to capture consistent changes and reduce the impact of transient fluctuations or overreporting.[30]
4. Participants were reassured about the confidentiality and anonymity of their responses to encourage honest reporting.
5. Data analysis included checks for outliers and inconsistencies to identify and control for potential reporting biases. [31]

Intervention Details

Each treatment session consisted of the following integrative manual therapy components:

Ligamentous Articular Strain Technique (LAST):

Participants are positioned comfortably to facilitate access to the knee joint. The therapist applies gentle, precise pressure to the knee capsule and related ligament structures to identify areas of restriction or abnormal tension. Using controlled passive movements, the therapist moves the joint in specific directions to release tension patterns and restore optimal joint alignment and mobility. These techniques target the medial and lateral collateral ligaments, the anterior and posterior cruciate ligaments, and the joint capsule.

Muscle Energy Technique (MET):

After LAST, MET is applied to the periarticular muscles that are often affected in KOA, including the quadriceps, hamstrings, and iliotibial band. Participants perform isometric contractions against the therapist's resistance for 5 to 10 seconds, followed by a relaxation period in which the therapist slowly stretches the targeted muscle. This cycle is repeated 3 to 5 times per muscle group to improve muscle length, strength, and neuromuscular control.

The combination of LAST and MET aimed to simultaneously address articular restrictions and muscular imbalances contributing to knee pain and dysfunction in KOA.

Data Analysis

Statistical analysis was performed using SPSS version 16.0. Descriptive statistics, including means and standard deviations, were calculated for demographic variables such as age, gender, and baseline clinical characteristics of the participants. [32] Given that the data did not meet the assumption of normality, non-parametric tests were employed. Specifically, the Friedman test was used to compare across three time points, baseline and post-intervention scores at the second and fourth weeks for primary outcome measures, including pain intensity (VAS), knee function (KOOS), range of motion (ROM), and functional mobility (Timed Up and Go test). [33]

Cohen's effect size (d) was calculated to quantify the magnitude of change between baseline and post-treatment outcomes. Effect sizes were interpreted as small (0.2), medium (0 [5]), or large (0.8) according to conventional thresholds. [34] The primary aim of this pilot study was to determine the effect size of the integrative Ligamentous Articular Strain Technique (LAST) and Muscle Energy Technique (MET) intervention on pain, function, and mobility in individuals with knee osteoarthritis. The level of significance was set at 0.05 to determine whether

statistically significant differences existed between baseline and post-intervention measurements.

All analyses were conducted on an intention-to-treat basis, and missing data were handled using the last observation carried forward (LOCF) method to preserve sample size and reduce bias. [35]

Results

Demographic characteristics of respondents

A total of thirty-two participants were screened for eligibility to participate in this study. After the screening process, twenty-eight subjects met the inclusion criteria and completed the LAST and MET intervention. Four subjects were excluded due to not meeting eligibility criteria or withdrawing consent prior to the intervention. [36] The demographic characteristics of the twenty-eight participants are summarized in Table 1. The sample consisted of both male and female participants, with an age range representative of typical knee osteoarthritis populations. Other variables such as body mass index (BMI), duration of symptoms, and affected knee side were also recorded. [37]

This demographic profile indicates a representative sample of middle-aged to older adults with mild to moderate knee osteoarthritis, suitable for evaluating the effects of the integrative manual therapy intervention

Primary outcomes: Adherence and Feasibility

A total of 28 participants who met the inclusion criteria and enrolled in this pilot study investigating the integrative application of LAST and MET for knee osteoarthritis completed the intervention with a 100% retention rate. Over the 4-week treatment period, all participants tolerated both LAST and MET interventions without experiencing any serious or adverse side effects. Five participants reported mild muscle soreness following the first MET session, and four participants described slight discomfort during

the initial LAST application; however, these symptoms were transient and did not hinder participation. The remaining participants reported feeling comfortable or relaxed during sessions. No dropouts occurred, and participant feedback was overwhelmingly positive. Twenty-one participants noted improvements in knee joint flexibility, reduced stiffness, and increased ease of movement after the first week. An additional seven participants reported similar effects by the end of the Four week. Overall, participants expressed satisfaction with the intervention and supported further implementation of this integrative therapeutic approach.

These findings align with previous literature suggesting that manual therapy techniques such as MET and LAST may improve joint mobility, reduce pain, and enhance functional outcomes in individuals with musculoskeletal conditions, including osteoarthritis. [38,39,40]. This preliminary data supports the feasibility and acceptability of LAST and MET in the management of knee osteoarthritis and provides a foundation for future larger-scale studies.

Secondary Outcomes: Effect of Integrative LAST and MET on Knee Pain and Functional Ability in Knee Osteoarthritis Patients

Table 2 shows the results of the Friedman test on knee pain and functional ability at three time points: baseline, after 2-week treatment, and after 4-week treatment. The analysis indicated statistically significant improvements across all measured parameters:

- 1) **Pain intensity** showed significant reduction from baseline (mean difference = 6.23 ± 1.01 , $p=0.001$), after 2-week treatment (mean difference = 3.89 ± 0.94 , $p=0.001$), and after 4-week treatment (mean difference = 1.71 ± 0.85 , $p=0.001$)
- 2) **Pain during activity** also decreased from baseline (mean difference = 5.85 ± 0.88 , $p=0.001$), after 2-week treatment (mean difference = 3.54 ± 0.81 , $p=0.001$), and after

4-week treatment (mean difference = 1.40 ± 0.73 , $p=0.001$)

- 3) **Stiffness score** decreased significantly from baseline (mean difference = 3.12 ± 0.79 , $p=0.001$), after 2-week (mean difference = 2.02 ± 0.64 , $p=0.001$), and after 4-week treatment (mean difference = 0.78 ± 0.60 , $p=0.001$)
- 4) **Functional ability**, measured by a modified WOMAC index, improved with a decrease in difficulty scores from baseline (mean difference = 5.47 ± 1.12 , $p=0.001$), after 2-week treatment (mean difference = 3.26 ± 0.96 , $p=0.001$), and after 4-week treatment (mean difference = 1.35 ± 0.71 , $p=0.001$).

These results suggest that the integrative application of LAST and MET has a significant effect in reducing pain, stiffness, and improving knee function in patients with knee osteoarthritis, even within a short intervention period.

Table 3 showed that changes in the quality of life of participants with knee osteoarthritis following the integrative LAST and MET intervention were statistically significant, as measured by the Friedman test. The results were as follows:

- 1) **Anxiety levels** before treatment (mean difference = 3.14 ± 0.58 , $p=0.001$), after 2-week treatment (mean difference = 1.93 ± 0.75 , $p=0.001$), and after 4-week treatment (mean difference = 1.31 ± 0.47 , $p=0.001$);
- 2) **Limitation of daily activities** before treatment (mean difference = 3.31 ± 0.47 , $p=0.001$), after 2-week treatment (mean difference = 2.07 ± 0.46 , $p=0.001$), and after 4-week treatment (mean difference = 1.03 ± 0.19 , $p=0.001$);
- 3) **Mobility and movement confidence** before treatment (mean difference = 3.45 ± 0.51 , $p=0.001$), after 2-week treatment (mean difference = 2.03 ± 0.42 , $p=0.001$), and after 4-week treatment (mean difference = 1.03 ± 0.19 , $p=0.001$);

- 4) **Knowledge and self-control over symptoms** before treatment (mean difference = 3.17 ± 0.71 , $p = 0.001$), after 2-week treatment (mean difference = 1.34 ± 0.48 , $p = 0.001$), and after 4-week treatment (mean difference = 1.00 ± 0.00 , $p = 0.001$)
- 5) **Work and productivity** before treatment (mean difference = 3.14 ± 0.58 , $p = 0.001$), after 2-week treatment (mean difference = 2.00 ± 0.38 , $p = 0.001$), and after 4-week treatment (mean difference = 1.07 ± 0.26 , $p = 0.001$).

Discussion

The pilot study aimed to determine the effect size of integrative LAST and MET interventions on knee pain and quality of life in adult individuals with KOA. With full adherence, positive participant feedback, and a 100% retention rate, our findings suggest that the integrative LAST and MET intervention was well received and potentially beneficial for this patient population. These results indicate that the combined osteopathic approach may serve as a promising complementary therapy in the management of KOA. [41] Based on participant feedback, several factors contributed to the high acceptance of the intervention. Firstly, comfort and ease during therapy were frequently cited. The gentle, hands-on, non-invasive nature of both LASTT and MET techniques provided a sense of relaxation and safety. Secondly, participants who experienced measurable improvements, such as reduced pain, greater ease in movement, and reduced stiffness, were more likely to view the intervention favorably. The sense of rapid, tangible progress reinforced participant confidence in the therapy. Importantly, no significant or serious side effects were reported throughout the intervention period, suggesting that this integrative approach is safe for short-term use in individuals with KOA. Furthermore, results demonstrated meaningful reductions in pain intensity, stiffness, and functional limitations, along with improved quality of life after just two

weeks of treatment. These findings are consistent with studies highlighting the efficacy of manual therapy in osteoarthritis management. [42,43]

Several mechanisms may explain the observed effects. LASTT is believed to correct dysfunctional joint and ligament tension by restoring neutral positioning, thereby enhancing joint function and reducing nociceptive input. [44] MET involves active patient participation through isometric contractions, leading to post-isometric relaxation and enhanced joint mobility. [45] Both techniques can improve proprioceptive input, reduce muscle hypertonicity, and optimize neuromuscular coordination, leading to symptom relief. [46]

In light of these promising outcomes, clinicians may consider incorporating LASTT and MET into conservative treatment plans for KOA, particularly for patients seeking non-pharmacological options. Future research should focus on larger, randomized controlled trials to evaluate the long-term efficacy, cost-effectiveness, and comparative outcomes of these manual therapies against standard care. Additionally, further studies exploring biomechanical and neurophysiological mechanisms would help clarify how these interventions affect joint function and pain pathways in OA patients [1,5]

The strength of this study lies in its focus on addressing the need for integrative manual therapy strategies in KOA, a condition that significantly impairs quality of life. By providing initial data on effect size and patient acceptance, the study adds valuable preliminary evidence to the field of osteopathic and rehabilitative medicine. However, this study is not without limitations. The small sample size, absence of a control group, and short duration may limit the generalizability of results. Moreover, the findings are context-specific and may not fully represent broader patient populations or clinical settings.

Nonetheless, this study offers valuable insights into the potential role of integrative LAST and MET in reducing KOA symptoms and improving patient well-being. These findings support further exploration of manual therapy as a viable and patient-friendly approach in the multidisciplinary management of osteoarthritis.

Conclusion

The results of this pilot study indicate that the integrative application of LAST and MET may have a positive impact on adult patients with knee osteoarthritis by reducing pain and stiffness while improving functional ability and quality of life. These findings suggest that this combined manual therapy approach offers a promising adjunctive treatment for individuals suffering from KOA, a condition that is often chronic and functionally limiting. [47]

The study demonstrated significant reductions in the severity and frequency of knee pain, improvements in mobility, and enhanced quality of life measures among participants who received the LAST and MET interventions. These outcomes provide preliminary evidence supporting the potential efficacy of osteopathic manual therapy techniques in addressing the physical impairments and psychosocial challenges commonly associated with KOA. [48,49]

The non-invasive, patient-friendly, and low-risk nature of LAST and MET makes them attractive therapeutic options for patients seeking non-pharmacological or complementary treatments for musculoskeletal conditions. However, it is important to acknowledge that this study had a small sample size and lacked a control group, and the long-term effects of these interventions remain to be fully established. Consequently, further large-scale randomized controlled trials are warranted to confirm these findings, better

understand the underlying mechanisms, and assess the sustained impact of integrative manual therapy on knee osteoarthritis. [50,51]

Nonetheless, the results provide a strong foundation for future investigations into the role of LAST and MET as viable components of multidisciplinary care strategies aimed at improving function, reducing pain, and enhancing quality of life for individuals with knee osteoarthritis.

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Competing interests

The authors declare that they have no competing interests.

Ethical clearance

The research had been approved by the Faculty Ethics Review Committee (FERC) of the Faculty of Health Sciences, UiTM Puncak Alam Campus (Approval ID: 500-FSK (PT. 23/4) and the ethics commission of the faculty of medicine, Universitas Mulawarman Samarinda. (Ethics No. 162/KEPK-FK/VIII/2023) and also received ethical approval from the Health Research Ethics Committee of the Health Polytechnic, Ministry of Health, East Kalimantan, Indonesia (Ethics DP.04.001/7.1/07762/2023). During the research, the researcher pays attention to the ethical principles of information to consent, respect for human rights, beneficence and non-maleficence.

Authors' contributions

S was accountable for the conceptualisation of the research, execution and management of the study protocol, data collection, preliminary analysis, and manuscript drafting. Research and manuscript supervision, review, and editing were the responsibility of AA and ZZ.

Table 1. Demographic Characteristics of Participants (n = 28)

Characteristic	Value
Age (years), mean ± SD	58.3 ± 7.4
Gender, n (%)	
Male	12 (42.9%)
Female	16 (57.1%)
Body Mass Index (BMI), mean ± SD	27.8 ± 3.5
Duration of Symptoms (months), mean ± SD	24.6 ± 10.2
Affected Knee, n (%)	
Right	15 (55.2%)
Left	13 (44.8%)
Kellgren-Lawrence Grade, n (%)	
Grade II	17 (62.1%)
Grade III	11 (37.9%)

Table 2. Changes in Pain Intensity, Activity Pain, Stiffness, and Functional Ability Across Timepoints (Baseline, Week 2, Week 4)

Outcome Measure	Baseline (Mean ± SD)	After Week 2 (Mean ± SD)	After Week 4 (Mean ± SD)	p-value
Pain Intensity (VAS 0–10)	6.23 ± 1.01	3.89 ± 0.94	1.71 ± 0.85	p < 0.001
Pain During Activity (0–10)	5.85 ± 0.88	3.54 ± 0.81	1.40 ± 0.73	p < 0.001
Stiffness Score (0–4)	3.12 ± 0.79	2.02 ± 0.64	0.78 ± 0.60	p < 0.001
Functional Difficulty (*KOOS subscale)	5.47 ± 1.12	3.26 ± 0.96	1.35 ± 0.71	p < 0.001

* KOOS: Knee injury and Osteoarthritis Outcome Score Statistical test: Friedman test; significance level set at $p < 0.05$.

Table 3. Changes in Quality of Life Parameters Across Timepoints (Baseline, Week 2, Week 4)

Quality of Life Domain	Baseline (Mean ± SD)	After Week 2 (Mean ± SD)	After Week 4 (Mean ± SD)	p-value
Anxiety	3.14 ± 0.58	1.93 ± 0.75	1.31 ± 0.47	0.001
Limitation of Daily Activities	3.31 ± 0.47	2.07 ± 0.46	1.03 ± 0.19	0.001
Mobility and Movement Confidence	3.45 ± 0.51	2.03 ± 0.42	1.03 ± 0.19	0.001
Knowledge & Control Over Symptoms	3.17 ± 0.71	1.34 ± 0.48	1.00 ± 0.00	0.001
Work and Productivity	3.14 ± 0.58	2.00 ± 0.38	1.07 ± 0.26	0.001

Statistical test: Friedman test; significance level set at $p < 0.05$.

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