

A Randomized Controlled Trial on the Combined Effect of Prolotherapy and Personalized Physical Activity on Knee Osteoarthritis Progression: Impact on IL-1 β , MMP-3 Biomarkers and Clinical Outcomes

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ABSTRACT

Background

Non-operative management of knee osteoarthritis (OA) remains a significant challenge, primarily focusing on pain relief, functional improvement, and inflammation reduction. Prolotherapy has been recognized as an effective treatment for joint and ligament pain, with evidence supporting its ability to improve outcomes in knee OA. Structured physical activity also offers benefits in terms of mobility and function, although its effects are less pronounced when used in isolation, without adjunctive therapy. In knee OA, inflammation is driven by biomarkers such as interleukin-1 beta (IL-1 β) and matrix metalloproteinase-3 (MMP-3), which contribute to cartilage degradation. While evidence supporting treatments that improve both clinical and biochemical outcomes is limited, research on combined intervention therapies and physical activity, capable of impacting both clinical outcomes and biomarkers, remains inconclusive.

Objective

This study aimed to determine the effects of prolotherapy combined with personalized physical activity on (1) clinical outcomes, (VAS-assessed pain intensity, WOMAC-assessed functional status, and quality of life) and on (2) serum IL-1 β and MMP-3 levels in patients with knee OA.

Methods

This randomized controlled trial employed a pretest–posttest nonequivalent control group design. A total of 74 outpatients with knee osteoarthritis (OA) who met the predefined inclusion criteria were recruited from the pain clinics of three hospitals affiliated with the Faculty of Medicine, Universitas Islam Indonesia. Participants underwent open-label randomization and were allocated to either the intervention or control arm. The intervention group received standardized prolotherapy combined with personalized physical activity. Clinical outcomes—functional status assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and health-related quality of life—as well as serum inflammatory biomarkers (interleukin-1 beta [IL-1 β] and matrix metalloproteinase-3 [MMP-3]) were measured at baseline and following the intervention. Statistical analysis was conducted using paired t-tests to evaluate within-group changes and analysis of covariance (ANCOVA) to assess adjusted between-group effects.

Results

Participants receiving prolotherapy combined with personalized physical activity demonstrated statistically significant reductions in pain and functional impairment, improved quality of life, and significant decreases in IL-1 β and MMP-3 levels compared with baseline and control outcomes, indicating simultaneous clinical and inflammatory modulation.

Conclusion

The combination of prolotherapy and personalized physical activity resulted in superior clinical improvement and meaningful reduction of inflammatory biomarkers associated with knee OA progression, supporting its potential as an integrative and mechanism-targeted therapeutic strategy.

Keywords

Knee Osteoarthritis; Prolotherapy; Exercise Therapy; Physical Activity; Inflammation; Biomarkers; Interleukin-1beta; Matrix Metalloproteinase 3; Pain Assessment

INTRODUCTION

Knee osteoarthritis (OA) is one of the most common degenerative disorders in older adults, affecting synovial joints and resulting in persistent pain, functional decline, and substantial economic burden¹. The knee is frequently involved and exhibits progressive articular cartilage loss with marginal osteophyte formation, subchondral bone sclerosis and cysts, low-grade synovitis, and capsular fibrosis that restricts range of motion². Diagnosis can be made clinically and supported by conventional radiography to grade structural change³ or by ultrasonography to characterize synovitis and osteophytes and to assist grading⁴. Globally, an estimated 528 million people lived with OA in 2019, with rising prevalence over time; women are affected more often than men, and nearly 45% of women over 65 years report symptoms, a pattern also observed in Indonesia (5–7). In primary care, knee OA incidence increased from 2.0% in 1996 to 3.6% in 2016 (8). The disease imposes physical and psychological harms and is a leading cause of disability, reduced mobility, and loss of independence, with major societal costs⁹.

Biomarkers provide a mechanistic lens on inflammatory and catabolic activity that drives symptoms and structural progression. Interleukin-1 β (IL-1 β) is a central upstream

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cytokine in OA pathogenesis; by binding the IL-1 receptor, it initiates pro-inflammatory signaling that promotes synovitis, cartilage matrix degradation, and pain generation^{10,11}. Cartilage breakdown fragments and enzymes—including CTX-II and matrix metalloproteinase-3 (MMP-3)—often rise in OA and correlate with inflammatory symptoms, pain, and structural damage^{12,13}. Overexpression of IL-1 β and MMP-3 has been implicated in early inflammatory changes and cartilage degradation; higher MMP-3 levels track with more severe radiographic disease¹³. Because these biomarkers change early, their measurement may facilitate earlier detection and provide prognostic information, complementing clinical and imaging assessments¹³⁻¹⁵.

Management includes a range of pharmacological interventions, interventional, and non-pharmacologic strategies. Nonsteroidal anti-inflammatory drugs, corticosteroids, opioids, and related analgesics can reduce pain transiently but do not modify disease course and carry risks, including metabolic, cardiovascular, bleeding, and dependence, when used long term; many patients remain symptomatic¹⁶⁻¹⁸. Interventional options such as intra-articular corticosteroid, hyaluronate, platelet-rich plasma, ozone, radiofrequency, and prolotherapy show heterogeneous effectiveness and practical trade-offs. Evidence suggests prolotherapy may deliver superior longer-term pain relief and functional improvement compared with corticosteroid, hyaluronate, ozone, or exercise alone, consistent with putative joint-stabilizing and nociceptive-modulating effects¹⁹⁻²¹. Technique, injection targets, and ultrasound guidance likely influence outcomes and may explain variability across trials²².

Non-pharmacologic care remains indispensable. Diet, weight reduction, hydrotherapy, biomechanical interventions, electrotherapy, diathermy, patient education, thermal modalities, and personalized physical activity provide additive benefit. Exercise programs consistently reduce pain and WOMAC scores and improve function; resistance training of lower-extremity musculature strengthens periarticular support and enhances joint stability²³⁻²⁵. Nevertheless, when compared head-to-head with interventional therapy, exercise alone can be less potent for short-term analgesia^{19,26}.

These converging lines of evidence motivate a mechanism-informed, integrative approach. Combining

prolotherapy with personalized, measurable physical activity may address complementary pathways—nociception, joint stability, and behavioral adaptation—while embedding biomarker assessment with IL-1 β and MMP-3 aligns clinical outcomes (VAS, WOMAC, quality of life) with inflammatory and catabolic modulation. This study therefore evaluates whether the combined strategy yields superior clinical improvement and favorable biomarker shifts compared with prolotherapy alone.

MATERIAL AND METHODS

Study design

This randomized controlled trial was approved by the Ethics Commission of the Faculty of Medicine, Public Health, and Nursing (FK-KMK) of Universitas Gadjah Mada, with approval number KE/FK/1000/EC/2024. Additionally, research permits were obtained from Madiun Regional General Hospital (RSUD Madiun), RSIY PDHI, and Ludira Husada General Hospital (RSU Ludira Husada) and the study was registered at ClinicalTrials.gov (NCT07355712). Informed consent was obtained from all participants. The study was conducted in accordance with the CONSORT reporting guidelines.

Sample Size and Clinical Data

The sample size for this study was calculated using the formula for unpaired numerical analytical samples, with the calculation performed using Stata software (power twomeans). The following parameters were used: alpha = 0.05, power = 0.80, and an allocation ratio of 1.

Based on quality-of-life data from a previous study by Sert et al. (2020), with a mean of 52.7 (SD = 9.1) in the intervention group and 45.9 (SD = 10.0) in the control group, the minimum required sample size was 33 participants per group. After adding 10% to account for potential dropout, the final sample size was determined to be 74 participants (27). A total of 84 participants were initially enrolled in the study, and 74 patients with knee osteoarthritis completed the follow-up process. All participants received treatment at the pain clinic of Madiun Regional General Hospital, RSIY PDHI, and Ludira Husada Hospital. These hospitals are affiliated with the Faculty of Medicine, Universitas Islam Indonesia. The data collection period for this study spanned from July 2024 to June 2025.

Inclusion criteria: Adults ≥ 18 years with primary knee

osteoarthritis (OA) diagnosed by ultrasonography who provided written informed consent. Participants were required to be cooperative and capable of complying with the study protocol. **Exclusion criteria:** A history of traumatic knee arthritis; knee OA associated with trauma, fracture, ankylosing spondylitis, or septic arthritis; major comorbidities significantly impairing quality of life (e.g., cancer, heart failure, renal failure, stroke); use of NSAIDs or systemic steroids within 1 week; intra-articular corticosteroid injection within 2 months; or current use of injectable or hormonal contraception. **Dropout criteria:** Protocol nonadherence; serious adverse events deemed unsafe to continue (e.g., joint infection); withdrawal of consent; repeated missed visits; death; or erroneous enrollment discovered after baseline.

Randomisation

This study used computer-generated randomization performed with Stata software to allocate participants into two groups. Sealed envelopes containing the allocation sequence were provided to both researchers and care providers. Participants were recruited consecutively. A single-blind design was applied, in which participants were unaware of the therapy they received. Each intervention was delivered individually in separate rooms and at different time points. On assessment days, physicians and laboratory technicians were blinded to group allocation. All data were collected by trained research assistants who were unaware of allocation status, using face-to-face interviews. An external staff member performed data entry to ensure that the statistician remained blinded during analysis. The randomization envelopes were opened only after the statistical analysis was completed.

Treatment Protocols

Participants were consecutively recruited and randomly allocated to either the PRL treatment group (prolotherapy only) or the PRL + AFP group (prolotherapy combined with personalized physical activity). This study employed a single-blind design in which the outcome assessor was blinded to group allocation, while both participants and the treating physician were aware of the assigned intervention. All patients who met the inclusion criteria received prolotherapy with a dose of 20 mL of 15% hypertonic dextrose solution and 2 mL of 2% lidocaine, administered every 2 weeks for a total of 6 weeks. The therapy was delivered to periarticular

regions and surrounding joint spaces, including around the ACL or MCL ligaments, the infrapatellar bursa, the iliotibial band tendon edge, and 5 mL intra-articularly, with ultrasound guidance over three injection sessions. The procedure was carried out under strict supervision, following sterilization protocols, and ultrasound guidance was used to ensure precision in the injections. At the same time, the PRL+AFP group, which received additional interventions beyond prolotherapy, also underwent physical therapy starting 2 days post-injection. Exercises were performed at home under the guidance of trained professionals, with educational videos provided to the patients. The interventions included additional activities, such as muscle strengthening exercises and joint range of motion training, including isometric and isotonic quadriceps strengthening, hamstring stretching, and knee range of motion exercises. These exercises were conducted with 10 repetitions per session, 3 sessions per day, 3 days per week. For the range of motion exercises, Patients were instructed to flex and extend the knee within a tolerable range, performing the movements consecutively. Patients could adjust the frequency of exercises based on their individual condition. All exercises were demonstrated to the patients in detail.

Evaluation Parameter

Physical activity improvement in patients was measured through periodic observations measured through periodic observations, with assessments conducted four weeks after the intervention using several parameters, including the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the Visual Analog Scale (VAS), and quality of life evaluations, all carried out through direct patient observation. The initial assessment, or pretest, was conducted prior to the intervention to evaluate the severity of osteoarthritis (OA) using ultrasonography (USG), as well as to measure joint stiffness, pain, and functionality through the WOMAC index. This assessment aimed to establish baseline data, which would serve as a reference for evaluating post-intervention improvements. A clinical evaluation was performed to determine the baseline or pretest values, which included an assessment of the patient's initial condition before the intervention began. The parameters used in this evaluation included the WOMAC score to assess OA symptoms such as pain, stiffness, and functional impairment, as well as quality

of life measurements using relevant instruments. This process ensured that observed changes after the intervention could be compared with the patient's baseline condition, thereby allowing for an evaluation of the effectiveness of the treatment or intervention provided. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used as a primary parameter to assess clinical outcomes in both the PRL and PRL+AFP groups. WOMAC scores were measured at baseline (pre-test) and immediately following the intervention (post-test) to evaluate changes in pain, stiffness, and functional limitations associated with knee osteoarthritis. This assessment provided a comprehensive evaluation of the therapeutic effects in both groups, enabling comparisons of improvements in joint function and pain relief between the prolotherapy-only (PRL) group and the combined prolotherapy and personalized physical activity (PRL+AFP) group. The use of WOMAC ensured a standardized and objective measure of clinical progress throughout the study

Biomarker

Peripheral venous blood samples were collected and analyzed both prior to treatment and 4 weeks after the intervention. A total of 5 mL of peripheral venous blood was drawn from each patient and transferred into serum separation tubes. The samples were allowed to clot at room temperature for 45 minutes, followed by centrifugation at 1000 x g for 15 minutes. The resulting serum was then separated and stored at -20°C for subsequent inflammatory biomarker analysis. The levels of interleukin-1 β (IL-1 β) and matrix metalloproteinase-3 (MMP-3) in the serum were determined using the enzyme-linked immunosorbent assay (ELISA) method. For the analysis, serum, Aquabides, and ELISA kits from ABclonal were used, with IL-1 β (Cat. No. RK00001, Lot No. 9680034260724) and MMP-3 (Cat. No. RK00308, Lot No. 9680000230724). In the ELISA procedure for measuring IL-1 β and MMP-3, a two-fold dilution of the samples was applied to antibody-coated microplate wells. After an incubation period of 2 hours at room temperature, the enzyme-conjugated secondary antibody was added and incubated for an additional 1-2 hours. Following five washes to remove non-bound materials, the chromogenic substrate specific to the enzyme was added for color development. The absorbance was then measured at 450 nm using a

microplate reader (Thermohet), providing quantitative data for IL-1 β and MMP-3 levels.

Statistical Analysis Methods Data analysis in this study was performed using statistical tests via STATA version 16.1. Descriptive statistics were employed to present sample characteristics, including frequency distributions, means, and standard deviations for each variable. Paired t-tests were used to assess differences within groups. Paired t-tests were applied to numeric variables to compare pre- and post-intervention data, including pain management, WOMAC scores, VAS, and changes in IL-1 β and MMP-3 levels. The Shapiro-Wilk normality test determines whether the data are normally distributed. A p-value larger than 0.05 suggests that the data are normally distributed. In our study, the normality of numerical variables was assessed using the Shapiro-Wilk test. The results showed that all key numerical variables, including IL-1 β , MMP-3, WOMAC scores (total, pain, function, stiffness), VAS, and utility values, had p-values greater than 0.05, indicating normal distribution. Therefore, the use of parametric tests such as paired t-tests and ANCOVA was appropriate. To adjust for pre-test differences, ANCOVA was employed for multivariate analysis, allowing for a more accurate comparison between the prolotherapy group and the prolotherapy combined with personalized physical activity group. This approach ensured the evaluation of clinical outcomes and biomarkers while accounting for baseline differences, with pre-test scores serving as covariates in the analysis.

RESULT

In this study, a total of 84 participants were initially recruited. After the screening process, 5 participants were excluded due to not meeting the inclusion criteria, resulting in 79 eligible participants at the start of the study. These 79 participants were then randomized into two groups: the control group (prolotherapy/PRL) and the intervention group (prolotherapy combined with personalized physical activity/PRL+AFP). Forty participants were assigned to the control group, and 39 participants were assigned to the intervention group. During the study, 3 participants from the control group and 2 participants from the intervention group experienced health. The detailed participant flow, including recruitment, exclusion, randomization, and reasons for dropout, is presented in Figure 1

Characteristics	Category	Group PRL	Group PRL+AFP	Sum		P value
				n	%	
Duration of pain	< 3 Months	3	5	8	10,81	0,454
	>3 Months	34	32	66	89,19	
OA Degrees Based on ultrasound	2	35	33	68	91,89	0,394
	3	2	4	6	8,11	
BMI	Not overweight	21	21	42	56,76	1,000
	Overweight	16	16	32	43,24	
Hypertension	Yes	13	14	27	36,49	0,809
	No	24	23	47	63,51	
Diabetes Mellitus (DM)	Yes	8	7	15	20,27	0,772
	No	29	30	59	79,73	
The Presence of Effusion	Yes	5	4	9	12,16	0,722
	No	32	33	65	87,84	

SD Standard deviation, BMI Body mass index, OA osteoarthritis, USG Mortada(2016) grading system, *Significance difference ($p < 0.05$)

Clinical outcomes and biomarkers of participants before and after treatment in patients

This study provides data comparing clinical outcome parameters and biomarkers before and after the intervention in both the control and intervention groups. As shown in Table 2, clinical outcomes and biomarkers were evaluated for both groups. In the control group, significant reductions were noted in several parameters, especially IL-1 β , which decreased from 92.64 pg/mL to 61.90 pg/mL ($p < 0.05$). This significant change indicates a notable reduction in inflammation, although the MMP-3 biomarker did not show significant changes ($p > 0.05$), with MMP-3 decreasing from 2.04 ng/mL to 1.62 ng/mL. The WOMAC total score showed more significant results, with the WOMAC pain score improving from 9.70 to 7.16 and the WOMAC function score improving from 42.43 to 28.70, indicating a substantial clinical improvement ($p < 0.05$). Additionally, the WOMAC stiffness parameter in the control group showed a significant decrease from 4.81 to 2.95, indicating a reduction in joint stiffness that contributed to improved patient mobility. Regarding quality of life parameters, both the VAS and utility scores

showed significant improvements post-intervention. The VAS score decreased significantly from 0.70 to 0.34, and the utility score increased from 0.67 to 0.74, with a significant p-value (0.0036), reflecting an improvement in patients' perceived quality of life as indicated by the utility score. In the intervention group, the observed results demonstrated greater changes compared to the control group. All parameters analyzed in the intervention group showed significant reductions, with p-values < 0.05 for IL-1 β , MMP-3, WOMAC total, and subcomponents of WOMAC such as pain, function, and stiffness. Interleukin-1 β showed a significant decrease from 106.02 pg/mL to 57.21 pg/mL ($p < 0.05$), indicating a greater reduction in inflammation in the PRL+AFP intervention group. The average reduction in the MMP-3 biomarker in the PRL+AFP intervention group decreased from 2.95 ng/mL to 2.38 ng/mL, although it was not statistically significant ($p > 0.05$). This suggests that the PRL+AFP intervention may help lower inflammatory biomarkers, which could be associated with improvement in joint conditions

Table 2. Comparison clinical outcomes and biomarkers in the two participant groups

Parameters	Baseline, mean $\pm$ SD	Post-intervention, mean $\pm$ SD	Change(Δ), mean $\pm$ SD	p-value (within-group) ^a	p-value (between-group) ^b
IL-1 β (pg/mL)					0,2613
Group PRL	92,64 $\pm$ 60,50	61,90 $\pm$ 57,95	30,74 $\pm$ 46,65	0,0003*	
Group PRL + AFP	106,03 $\pm$ 70,73	57,21 $\pm$ 43,87	48,82 $\pm$ 60,43	<0,0001*	
MMP-3 (ng/mL)					0,0476*
Group PRL	2,04 $\pm$ 1,42	1,62 $\pm$ 0,98	0,42 $\pm$ 1,77	0,1598	
Group PRL + AFP	2,95 $\pm$ 1,93	2,38 $\pm$ 1,76	0,57 $\pm$ 2,43	0,1621	
WOMAC total					0,0089*
Group PRL	57,49 $\pm$ 16,72	38,57 $\pm$ 15,16	18,92 $\pm$ 11,20	<0,0001*	
Group PRL + AFP	50,32 $\pm$ 17,48	28,30 $\pm$ 11,30	22,03 $\pm$ 18,11	<0,0001*	
WOMAC Pain					0,0118*
Group PRL	9,70 $\pm$ 3,64	7,16 $\pm$ 3,25	2,54 $\pm$ 1,30	<0,0001*	
Group PRL + AFP	9,00 $\pm$ 3,58	5,78 $\pm$ 2,96	3,22 $\pm$ 1,84	<0,0001*	
WOMAC Function					0,0152*
Group PRL	42,43 $\pm$ 12,19	28,70 $\pm$ 12,21	13,73 $\pm$ 10,15	<0,0001*	
Group PRL + AFP	37,35 $\pm$ 14,34	21,08 $\pm$ 9,75	16,27 $\pm$ 16,95	<0,0001*	
WOMAC Stiffness					<0,0001*
Group PRL	4,81 $\pm$ 1,88	2,95 $\pm$ 1,61	1,86 $\pm$ 0,95	<0,0001*	
Group PRL + AFP	3,86 $\pm$ 2,29	1,43 $\pm$ 1,19	2,43 $\pm$ 1,82	<0,0001*	
Quality Of Life					
VAS/pain					0,9183
Group PRL	0,70 $\pm$ 0,18	0,34 $\pm$ 0,16	0,36 $\pm$ 0,13	<0,0001*	
Group PRL + AFP	0,65 $\pm$ 0,19	0,31 $\pm$ 0,16	0,33 $\pm$ 0,19	<0,0001*	
Utility					0,0215*
Group PRL	0,67 $\pm$ 0,11	0,74 $\pm$ 0,10	-0,07 $\pm$ 0,14	0,0036*	
Group PRL + AFP	0,68 $\pm$ 0,13	0,79 $\pm$ 0,10	-0,11 $\pm$ 0,14	<0,0001*	

SD Standard deviation; *: Significance difference ($p < 0.05$, ^apaired t test, ^banalysis of covariance (covariate) test

Table 2. Comparison of clinical and biomarker responses between the control group (PRL monotherapy) and intervention group (PRL+AFP combination). Within-group analyses: Both treatment arms demonstrated significant improvements from baseline across most clinical outcomes and biomarkers, confirming the effectiveness of both approaches in reducing symptoms and modulating biological responses. Between-group analyses: The PRL+AFP combination showed superior efficacy compared to PRL monotherapy, particularly for total WOMAC score ($p=0.0089$), WOMAC function ($p=0.0153$), WOMAC stiffness ($p<0.001$), and utility score ($p=0.0215$). Additionally, the combination led to a greater reduction in MMP-3 levels ($p=0.0476$), reflecting enhanced inhibition of extracellular matrix degradation. Non-significant between-group differences: Both treatments effectively reduced VAS pain scores and IL-1 β levels; however, no statistically significant differences were observed between groups (VAS: $p=0.9183$; IL-1 β : $p=0.2613$), indicating that the addition of AFP to PRL does not provide extra benefit for these outcomes. Safety monitoring. All participants were closely monitored by the research team throughout the observation period for pain complaints, need for paracetamol analgesia, and any adverse reactions. No adverse effects were reported. Only ten participants required a single 500 mg dose of paracetamol, which was taken on the second day.

DISCUSSION

Pain, as a critical parameter for assessing therapeutic success in knee osteoarthritis (OA), remains a primary issue in treatment management. The pain experienced by patients is often the leading reason individuals seek medical intervention, as it significantly impacts their quality of life and functional capacity. Prolotherapy has emerged as an effective therapeutic approach to alleviate pain in OA conditions. The combination of PRL + AFP intervention can offer a synergistic effect in non-surgical knee OA management, where prolotherapy helps reduce pain, inflammation, and stimulates the body's natural healing processes, while personalized physical activity strengthens muscles and improves joint mobility. Although both interventions provide significant individual benefits, their combination appears to deliver more optimal results in managing knee OA. This aligns with the OARSI recommendations, which emphasize structured exercise as a cornerstone of non-surgical knee OA therapy (23). Personalized physical activity plays a

crucial role in knee OA management as it can strengthen the muscles around the joint, improve flexibility, and reduce the load on the affected joint. In this study, the personalized physical activity intervention, consisting of quadriceps strengthening exercises and flexibility training, is expected to reduce the load on the knee joint and enhance its function. Recent meta-analysis findings show that aerobic exercises, such as Pilates, are most effective in improving pain and function (28), while both isokinetic and isotonic resistance exercises provide significant benefits (24). A study by Huang et al. (2024) suggests that moderate-intensity recreational physical activities, such as cycling, have a protective effect against OA risk, whereas low-intensity occupational physical activity may increase OA risk. These findings underscore the importance of selecting appropriate types and intensities of physical activity for individuals with OA (29), with combined interventions likely yielding better outcomes. In this study, pain assessment was performed using the commonly used Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). This instrument has been proven to be valid and reliable in measuring pain intensity and physical function in OA patients. The findings of this study indicate that participants receiving the PRL + AFP combination intervention were more effective in reducing pain, as measured by WOMAC pain, WOMAC function, and WOMAC stiffness, compared to participants who only received PRL. These results are consistent with previous studies, which showed that prolotherapy provides therapeutic benefits in reducing pain in knee OA (19,30), particularly in patients with grade 2 OA. A study by Dumais et al. (2013) also reported that participants receiving the combination therapy initially showed a significant reduction in pain compared to those who started with exercise and received prolotherapy later (26). Interestingly, the combination of prolotherapy with personalized physical activity showed better results compared to prolotherapy alone, emphasizing the important role of physical activity in enhancing the effects of prolotherapy. Physical activity helps improve muscle strength and joint stability, reducing the load on the knee joint and alleviating pressure on the inflamed tissues.

The quality of life of knee osteoarthritis (OA) participants was assessed using the EQ-5D-5L instrument before and after the intervention. This instrument provides an overview of participants' quality of life from the perspective of VAS and utility, both of

which have been validated in Indonesia. Analysis of the EQ-5D-5L results showed significant improvements across all domains in both the control and intervention groups. However, participants receiving the PRL+AFP intervention showed a more pronounced improvement in quality of life compared to those who only received PRL, particularly in the domains of mobility, usual activities, and anxiety/depression. This aligns with findings from Lim et al. (2020), which reported that structured physical exercise improves the psychosocial aspects of OA patients by enhancing self-confidence and functional independence (31). The multimodal approach showed better outcomes than a single approach because it combines physical improvements with support for mental health and holistic quality of life in knee OA patients. Significant improvements in domains such as daily activities, mobility, and anxiety/depression in this study reflect the holistic effects of the multimodal intervention, similar to findings from Huang (2024), who noted that physical activity was correlated with overall quality of life changes in knee OA patients (29). Based on the findings of Amin et al. (2021), physical activity is recommended for musculoskeletal disorders due to its effectiveness, comparable long-term outcomes to surgery, and its ability to prevent disease progression (32). A growing body of evidence, including the findings of Rajalaxmi et al. (2021), supports the use of structured personal physical activity as a first-line, non-invasive, and cost-effective strategy for managing adhesive capsulitis, and by extension, other musculoskeletal disorders characterized by pain and restricted mobility. In particular, dynamic exercise protocols combined with stretching have demonstrated notable efficacy (33). This therapeutic rationale extends to patients with knee osteoarthritis who experience joint stiffness and functional limitations. In such cases, interventions incorporating quadriceps strengthening, range of motion exercises, and stretching of the hamstrings and quadriceps muscles have been consistently associated with superior functional outcomes, including enhanced walking capacity, easier stair climbing, improved sit-to-stand transitions, and a reduced likelihood of falls. The findings of this study, in conjunction with previous multimodal research, demonstrate that the combination of PRL + AFP intervention is effective in improving the quality of life of knee OA patients. Findings from this study indicate that participants receiving PRL + AFP demonstrated a reduction in inflammatory biomarkers. The mechanism underlying this reduction

in inflammation can be attributed to decreased pro-inflammatory cytokine activity—an effect well documented in previous research—and possibly to increased activity of anti-inflammatory mediators. Personalized physical activity, as part of the combined prolotherapy and AFP intervention, aims to strengthen the muscles surrounding the knee joint, thereby reducing joint load and decreasing inflammatory stimulation. The significant reduction in IL-1 β observed in both groups supports the anti-inflammatory effects of prolotherapy. However, no statistically significant difference was found between the two groups indicating that the addition of AFP to PRL did not provide additional IL-1 β suppression.. In contrast, MMP-3 decreased significantly only in the intervention group. This finding can be explained by the synergistic effects between the injection—which reduces inflammation and promotes tissue remodeling—and physical activity, which stimulates mechanical improvement and metabolic modulation of the tissue. In a study conducted by Topol et al. (2022), alterations in neurocytokine levels and MMP-3 biomarkers were observed in grade-IV pain profiles following dextrose prolotherapy. Additionally, experimental studies on animal models have shown that intra-articular lubrication fluid can directly reduce the expression of both IL-1 β and MMP-3, highlighting its potential in modulating inflammatory markers in joint tissue (34,35). Taken together, the substantial reduction in IL-1 β observed in both groups, coupled with the significant decrease in MMP-3 only in the intervention group, can be explained by the anti-inflammatory effects of prolotherapy, which reduces the expression of pro-inflammatory cytokines such as IL-1 β (36). Personalized physical activity may offer additional benefits by inhibiting the degradation of the extracellular matrix through the regulation of MMP-3. Previous research has shown that prolotherapy can modulate inflammatory biomarkers associated with OA, including pro-inflammatory cytokines (34). The present findings further support these results, providing strong evidence that the combination of physical activity and prolotherapy enhances the regulation of harmful biomarkers such as MMP-3.

CONCLUSION

The combination of prolotherapy and personalized physical activity demonstrated significant improvements in key outcomes, including a reduction in MMP-3 levels, pain alleviation,

enhanced functional capacity, and improvements in stiffness and health-related quality of life (utility), compared to the group receiving only prolotherapy. These findings suggest that the synergistic effects of prolotherapy and personalized physical activity may offer a more comprehensive approach to managing knee osteoarthritis. Despite these promising results, no significant differences were observed between the groups in terms of the like IL-1 β biomarker or quality of life as measured by the Visual Analog Scale (VAS), indicating that further investigation of these biomarkers is necessary to fully understand their role in treatment outcomes. Moreover, this study emphasizes the importance of considering both biological markers and functional outcomes when evaluating the effectiveness of combination therapies. Overall, this research provides valuable insights into the potential benefits of integrating prolotherapy with personalized physical activity in the management of knee osteoarthritis, and it suggests directions for future research aimed at refining therapeutic strategies for this prevalent condition.

REFERENCES

- World Health Organization (WHO). Osteoarthritis. Overv Osteoarthritis [Internet]. 2023;1. Available from: <https://www.who.int/news-room/fact-sheets/detail/osteoarthritis>
- Bravatà V, Minafra L, Forte GI, Cammarata FP, Saporito M, Boniforti F, et al. DVWA gene polymorphisms and osteoarthritis. *BMC Res Notes*. 2015;**8**(1). <https://doi.org/10.1186/s13104-015-0987-1>
- Biscaldi E, Barra F, Evangelisti G, Ferrero S. Radiological assessment. Endometrial Cancer: Risk Factors, Management and Prognosis. 2018.
- Mortada M, Zeid A, Abd El-Hamid Al-Toukhy M, Ezzeldin N, Elgawish M. Reliability of a proposed ultrasonographic grading scale for severity of primary knee osteoarthritis. *Clin Med Insights Arthritis Musculoskelet Disord*. 2016 Jul 24;**9**:161-6. <https://doi.org/10.4137/CMAMD.S38141>
- Liu C xia, Gao G, Qin X qun, Deng C qing, Shen X jie. Correlation Analysis of C-terminal telopeptide of collagen type II and Interleukin-1 β for Early Diagnosis of Knee Osteoarthritis. *Orthop Surg [Internet]*. 1st ed. 2020;**12**(1):286-94. Available from: <https://www.who.int/news-room/fact-sheets/detail/osteoarthritis> <https://doi.org/10.1111/os.12586>
- Pal CP, Singh P, Chaturvedi S, Pruthi KK, Vij A. Epidemiology of knee osteoarthritis in India and related factors. *Indian J Orthop*. 2016 Sep 1;**50**(5):518-22. <https://doi.org/10.4103/0019-5413.189608>
- Sananta P, Qurotu'ain NA, Widasmara D, Noviya E. Correlation between Grade of Knee Osteoarthritis with Quality of Life of Patient in Secondary Referral Hospital in Indonesia. *Open Access Maced J Med Sci*. 2022 Apr 12;**10**(B):993-6. <https://doi.org/10.3889/oamjms.2022.9326>
- Spitaels D, Mamouris P, Vaes B, Smeets M, Luyten F, Hermens R, et al. Epidemiology of knee osteoarthritis in general practice: a registry-based study. *BMJ Open*. 2020 Jan 20;**10**(1). <https://doi.org/10.1136/bmjopen-2019-031734>
- Litwic A, Edwards MH, Dennison EM, Cooper C. Epidemiology and burden of osteoarthritis. *Br Med Bull [Internet]*. 2013 [cited 2024 Feb 12];**105**(1):185-99. Available from: <https://academic.oup.com/bmb/article/105/1/185/271633> <https://doi.org/10.1093/bmb/lds038>
- Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Vol. 2014, Mediators of Inflammation. Hindawi Publishing Corporation; 2014. <https://doi.org/10.1155/2014/561459>
- Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. *Nature Reviews Rheumatology*. 2010; **6**: p. 625-35. <https://doi.org/10.1038/nrrheum.2010.159>
- Liu C xia, Gao G, Qin X qun, Deng C qing, Shen X jie. Correlation Analysis of C-terminal telopeptide of collagen type II and Interleukin-1 β for Early Diagnosis of Knee Osteoarthritis. *Orthop Surg*. 2020;**12**(1):286-94. <https://doi.org/10.1111/os.12586>
- Henrotin Y. Osteoarthritis in year: biochemical markers. *Osteoarthritis and Cartilage*. 2021.11.001rkers. 2022;**30**: Osteoarthritis and Cartilage. W.B. Saunders Ltd; 2022. p. 237-48. <https://doi.org/10.1016/j.joca.2021.11.001>
- Chen JJ, Huang JF, Du WX, Tong PJ. Expression and significance of MMP3 in synovium of knee joint at different stage in osteoarthritis patients. *Asian Pac J Trop Med [Internet]*. 2014;**7**(4):297-300. Available from: [http://dx.doi.org/10.1016/S1995-7645\(14\)60042-0](http://dx.doi.org/10.1016/S1995-7645(14)60042-0) [https://doi.org/10.1016/S1995-7645\(14\)60042-0](https://doi.org/10.1016/S1995-7645(14)60042-0)
- Mailhot B, Christin M, Tessandier N, Sotoudeh C, Brethau F, Turmel R, et al. Neuronal interleukin-1 receptors mediate pain in chronic inflammatory diseases. *J Exp Med*. 2020 Sep 7;**217**(9). <https://doi.org/10.1084/jem.20191430>
- Scott DL, Berry H, Capell H, Coppock J, Daymond T, Doyle D V., et al. The long-term effects of non-steroidal anti-inflammatory drugs in osteoarthritis of the knee: A randomized placebo-controlled trial. *Rheumatology*. 2000;**39**(10):1095-101. <https://doi.org/10.1093/rheumatology/39.10.1095>
- Abramoff B, Caldera FE. Osteoarthritis: Pathology, Diagnosis, and Treatment Options. *Med Clin North Am*. 2020 Mar 1;**104**(2):293-311. <https://doi.org/10.1016/j.mcna.2019.10.007>
- Da Costa BR, Pereira T V., Saadat P, Rudnicki M, Iskander SM, Bodmer NS, et al. Effectiveness and safety of non-steroidal anti-inflammatory drugs and opioid treatment for knee and hip osteoarthritis: Network meta-analysis. *BMJ [Internet]*. 2021 Oct 12 [cited 2024 Feb 1];**375**. Available from: <https://pubmed.ncbi.nlm.nih.gov/34642179/>
- Rabago D, Patterson JJ, Mundt M, Kijowski R, Grettie J, Segal NA, et al. Dextrose prolotherapy for knee osteoarthritis: A randomized controlled trial. *Ann Fam Med [Internet]*. 2013 [cited 2023 Oct 9];**11**(3):229-37. Available from: <http://www.vhikits.com/Default.aspx> <https://doi.org/10.1370/afm.1504>
- Zeng CY, Zhang ZR, Tang ZM, Hua FZ. Benefits and Mechanisms of Exercise Training for Knee Osteoarthritis. Vol. 12, Frontiers in Physiology. Frontiers Media S.A.; 2021. <https://doi.org/10.3389/fphys.2021.794062>

21. Topol GA, Podesta LA, Reeves KD, Giraldo MM, Johnson LL, Grasso R, et al. Chondrogenic Effect of Intra-articular Hypertonic-Dextrose (Prolotherapy) in Severe Knee Osteoarthritis. *PM R*. 2016 Nov 1;8(11):1072-82. <https://doi.org/10.1016/j.pmrj.2016.03.008>
22. Hassan F, Trebinjac S, Murrell WD, Maffulli N. The effectiveness of prolotherapy in treating knee osteoarthritis in adults: A systematic review. *Br Med Bull* [Internet]. 2017 [cited 2024 Jan 30];122(1):91-108. Available from: <https://academic.oup.com/bmb/article/122/1/91/3061873> <https://doi.org/10.1093/bmb/ldx006>
23. Bannuru RR, Osani MC, Vaysbrot EE, Arden NK, Bennell K, Bierma-Zeinstra SMA, et al. OARSI guidelines for the non-surgical management of knee, hip, and polyarticular osteoarthritis. *Osteoarthr Cartil* [Internet]. 2019;27(11):1578-89. Available from: <https://doi.org/10.1016/j.joca.2019.06.011> <https://doi.org/10.1016/j.joca.2019.06.011>
24. Hislop A, Collins NJ, Tucker K, Semciw AI. The association between hip strength, physical function and dynamic balance in people with unilateral knee osteoarthritis: A cross-sectional study. *Musculoskelet Sci Pract*. 2023 Feb 1;63. <https://doi.org/10.1016/j.msksp.2022.102696>
25. Hinman RS, Hall M, Comensoli S, Bennell KL. Exercise & Sports Science Australia (ESSA) updated Position Statement on exercise and physical activity for people with hip/knee osteoarthritis. *J Sci Med Sport*. 2023 Jan 1;26(1):37-45. <https://doi.org/10.1016/j.jsams.2022.11.003>
26. Dumais R, Benoit C, Dumais A, Babin L, Bordage R, de Arcos C, et al. Effect of Regenerative Injection Therapy on Function and Pain in Patients with Knee Osteoarthritis: A Randomized Crossover Study. *Pain Med (United States)*. 2012;13(8):990-9. <https://doi.org/10.1111/j.1526-4637.2012.01422.x>
27. Sert AT, Sen EI, Esmailzadeh S, Ozcan E. The Effects of Dextrose Prolotherapy in Symptomatic Knee Osteoarthritis: A Randomized Controlled Study. *J Altern Complement Med*. 2020 May 1;26(5):409-17. <https://doi.org/10.1089/acm.2019.0335>
28. Luo Y, Chen X, Gong H, Chen L, Zhang L, Li S. Efficacy of aerobic exercises for knee osteoarthritis: a network meta analysis of randomized clinical trials. *J Orthop Surg Res*. 2025;20(1):557. <https://doi.org/10.1186/s13018-025-05973-z>
29. study on the association between types of physical activity, physical activity levels, and the incidence of osteoarthritis in adults: the NHANES 2007-2020. *Sci Rep* [Internet]. 2024;14(1):1-12. Available from: <https://doi.org/10.1038/s41598-024-71766-9> <https://doi.org/10.1038/s41598-024-71766-9>
30. Waluyo Y, Bukhari A, Adnan E, Artika SR, Yasin A, Wahyuni IN, et al. Association between cartilage biomarker level and functional outcome in knee osteoarthritis patients receiving dextrose prolotherapy: a cross-sectional study. *Bali Med J*. 2022;11(3):1151-6. <https://doi.org/10.15562/bmj.v11i3.3395>
31. Lim J, Choi A, Kim B. The Effects of Resistance Training on Pain , Strength , and Function in Osteoarthritis : Systematic Review and. *J Pers Med*. 2024;1-11. <https://doi.org/10.3390/jpm14121130>
32. Amin, J., Gohir, S., Kaiser, U., Siddiqui, A. A., Alshammary, F., & Alam MK. Original Article. *Bangladesh J Med Sci* [Internet]. 20(02):409-13. Available from: <https://www.banglajol.info/index.php/BJMS/article/view/51557> <https://doi.org/10.3329/bjms.v20i2.51557>
33. Rajalaxmi, V., Vasanthi, S., Sathya, S., Kirupa, K., Mary, S. D., Aravind, V., & Muthukumaran N. Original Article. *Bangladesh J Med Sci* [Internet]. 2021;20(02). Available from: <https://www.banglajol.info/index.php/BJMS/article/view/51550> <https://doi.org/10.3329/bjms.v20i2.51550>
34. Topol GA, Pestalardo IG, Reeves KD, Elias F, Steinmetz NJ, Cheng AL, et al. Dextrose Prolotherapy for Symptomatic Grade IV Knee Osteoarthritis: A Pilot Study of Early and Longer-Term Analgesia and Pain-Specific Cytokine Concentrations. *Clin Pract*. 2022 Dec 1;12(6):926-38. <https://doi.org/10.3390/clinpract12060097>
35. Zhu L, Lin W, Kluzek M, Miotla-Zarebska J, Batchelor V, Gardiner M, et al. Liposomic lubricants suppress acute inflammatory gene regulation in the joint in vivo. *Acta Biomater*. 2025;198:366-76. <https://doi.org/10.1016/j.actbio.2025.04.022>
36. Danto SI, Shojaei N, Singh RSP, Li C, Gilbert SA, Manukyan Z, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of PF-06650833, a selective interleukin-1 receptor-associated kinase 4 (IRAK4) inhibitor, in single and multiple ascending dose randomized phase 1 studies in healthy subjects. *Arthritis Res Ther*. 2019;21(1):1-17. <https://doi.org/10.1186/s13075-019-2008-6>