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Specific Biomarker Level and Function Outcome Changes in Treatment of Patients with Frozen Shoulder Using Dextrose Prolotherapy Injection

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***Abstract*—**Frozen shoulder (FS) is an insidious, painful condition caused by an inflammatory condition that causes fibrosis of the glenohumeral joint capsule, which causes progressive stiffness and restriction of the active and passive range of motion (ROM) of the shoulder. The studies of FS are still limited. This single-blinded randomized controlled trial involved participants with FS. The study participants were divided into two groups. The Prolotherapy group was the study group, and the Normal Saline (NS) group was the control group. Both groups were given injections at weeks 0, 2, 4, and 6. Matrix Metalloproteinase-1 (MMP-1) and Tissue Inhibitor Metalloproteinase-1 (TIMP-1) were measured at week six and week 12 after the last injection. The Disabilities of The Arm, Shoulder, and Hand (DASH) Score and ROM were measured at weeks 0, 2, 4, and 6 before and after injection and week 12. Comparative analysis was performed using repeated measures Paired T-Test, and data processing to assess correlation was using ANOVA. The result showed a significant decrease in The Disability of the Arm, Shoulder, and Hand (DASH) score in prolotherapy injection patients in each measurement week ($p < 0.05$). While the measurement of ROM, each direction of shoulder motion showed a significant difference in average each week, from week 0 to week 6 ($p < 0.05$). Dextrose prolotherapy injection results significantly improved the functional outcome of the shoulder joint and ROM. They did not show significant results in assessing the specific biomarker, MMP-1, and TIMP-1, in tissue repair. This study suggests an alternative to injection prolotherapy in FS patients; it has minimal adverse effects and is efficient in time and cost.

***Keywords*—**Frozen Shoulder, ROM, DASH Score, prolotherapy, MMP-1, TIMP-1.

I. Introduction

FROZEN shoulder (FS) is a common musculoskeletal disease that causes significant morbidity. It is characterized by functional restriction of both active and passive shoulder motion, caused by an inflammatory condition that causes the glenohumeral joint capsule to become fibrosis [1], [2]. Matrix Metalloproteinase-1 (MMP-1) and Tissue Inhibitor Metalloproteinase-1 (TIMP-1) are specific biomarkers used to diagnose and evaluate the fibrosis breakdown process in the glenohumeral joint capsules of FS [3], [4]. In addition, one of the conditions that cause the most substantial morbidity and has an undetermined most effective treatment is FS. Several options for treatment are currently being developed to treat FS [5]. To overcome the issues associated with conventional modalities, therapeutic approaches have been developed and implemented in FS [6], one of which is dextrose prolotherapy injection. Prolotherapy is an injection therapy that addresses certain compounds in the articular spaces, ligaments, and tendons that causes pain and disability around joint spaces that stimulate a proliferation cascade to enhance tissue repair and strength [7]. Dextrose prolotherapy injection has an excellent potential to enhance the functional outcome and reduce the pain of FS [8]; besides reporting the patient's functional outcome, we also examined the effects of prolotherapy in tissue repair by assessing the specific biomarker, MMP-1 and TIMP-1.

Aim

We aimed to determine the effectiveness of dextrose prolotherapy injection to improve functional outcome (ROM and DASH score), along with MMP-1, TIMP-1 and levels of MMP-1/TIMP-1 ratio as an indicator of tissue repair in glenohumeral joint among FS patients.

II. Material and Methods

This study is an experimental research that uses a double-blind, randomized controlled trial. In the single-arm prospective study, participants were eligible based on predetermined criteria. Inclusion criteria were: patients aged 30-65 years; and diagnosis of FS by anamnesis with physical examination restriction of ROM with chronic symptoms (> 3 months); with exclusion criteria: contraindication to prolotherapy like inflammation of shoulder. Dextrose prolotherapy injection in the rotator cuff, intraarticular glenohumeral joint, long head tendon biceps, and acromioclavicular joint injections with 15% dextrose, respectively, were injected at week 2, week 4, and week 6 for 12 weeks observation. The participants were then monitored for 12 weeks. The specific biomarker MMP and TIMP, ROM, and DASH score (assess the quality of available repair of the shoulder joint) were measured at baseline, at week six, and week 12. Baseline MMP-1 and TIMP-1 levels were evaluated using an enzyme-linked immunoassay (ELISA) procedure. Research data were analyzed. Statistically, comparative analysis differentiated the treatment groups performed by ANOVA Test and data processing to assess the

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correlation between therapy efficacy and functional outcome using Paired T-Test analysis. The ethical approval was received from the ethical committee in the Faculty of Medicine at Hasanuddin University.

III. Results

A. Characteristics of Patients

This study has 15 subjects diagnosed with FS and injected using prolotherapy into the shoulder joint. A total of two patients experienced dropout at the time of follow-up treatment. The patient's main complaint is pain in the shoulder and limitation of motion for 1-5 months. Each patient injected has previously measured the DASH Score and ROM. Patients consist of 40% male and 60% female. The average age of prolotherapy patients is 59 years, ranging from 50-59 years.

The patient's ROM was measured before and after every week of injection, while the DASH Score was measured every time the patient came for injection control. Injections were performed at week 0, week 2, and week 6. At week 12, the patient was only measured for ROM and DASH scores. Meanwhile, blood serum collection for MMP-1 and TIMP-1 measurements was carried out at week 0, week 6, and week 12.

In Table I, serum levels of MMP-1 at week 0 (baseline) and week 6 at the end of the injection showed an average increase in weeks 0-6 ((6.75 (1.58) versus 6.13 (8.13)), as well as at 6-12 weeks ((7.24 (1.98) versus 6.75 (1.58)). However, statistical calculations showed no significant difference between serum levels at week 0 and week 6 ($p > 0.05$), as well as serum levels from week 0 to week 12 ($p > 0.05$).

TIMP-1 serum levels at week 0 and week 6 showed an increase ((233.31 (287.28) versus 274.78 (113.55)); however, TIMP-1 serum levels showed a decrease at week 12 compared to week 6 ((257.43 (110.85) versus 274.78 (113.55)). While the statistical calculation of serum levels between week 0 and week 6 and between week 6 and week 12 did not show a significant difference ($p > 0.05$).

The ratio of MMP-1/TIMP-1 serum levels at week 0 and week 6 also showed an increase ((0.022 (0.02) versus 0.024 (0.01)), an increase was also shown at week 12 ((0.027 (0.01)). 01) versus 0.024 (0.01). While weekly statistical calculations did not show a difference in the mean serum levels ($p > 0.05$).

TABLE I
SERUM LEVELS OF MMP-1 AND TIMP-1

Parameter	Baseline	Week 6	P-value of Baseline - Week 6	Week 12	P-value of Week 6 - Week 12	P-value of Baseline - Week 12	P-value of Baseline - Week 6 - Week 12
	Median (IQR)	Median (IQR)		Median (IQR)			
MMP-1	6.13 (8.13)	6.75 (1.58)	0.496	7.24 (1.98)	0.173	0.256	0.247
TIMP-1	233.31 (287.28)	274.78 (113.55)	0.57	257.43 (110.85)	0.245	0.65	0.344
MMP-1/TIMP-1	0.022 (0.02)	0.024 (0.01)	0.91	0.027 (0.01)	0.125	0.14	0.282

TABLE II
DASH SCORE AND ROM

Parameter	Baseline	Week 2	Week 4	Week 6	P-value Baseline - Week 6	Week 12	P-value Baseline - Week 12
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		Mean ± SD	
DASH Score	56.36 ± 12.08	35.5 ± 13.28	24.367 ± 13.01	14.073 ± 10.82	0.0001	8.753 ± 7.94	0.001
				Mean ROM			
Flexion	134.83 ± 18.36	143.83 ± 18.58	157.67 ± 19.74	159.5 ± 22.06	0.0001	156.33 ± 28.63	0.001
Extension	38 ± 12.96	47 ± 10.01	51.67 ± 9.94	55.67 ± 8.58	0.0001	55 ± 10.35	0.0001
Abduction	129.17 ± 17.87	136 ± 29.27	152.5 ± 24.65	155.33 ± 24.65	0.02	158.33 ± 23.954	0.003
Adduction	53.17 ± 24.72	62.83 ± 16.95	62.5 ±11.65	63.5 ± 7.18	0.229	59 ± 9.85	0.745
Internal Rotation	46.67 ± 18.21	51.5 ± 21.71	61.3 ± 22.69	60.5 ± 20.44	0.227	73 ± 13.86	0.004
External Rotation	59.833 ± 23.42	67 ± 17.75	70.83 ± 16.68	74 ± 13.52	0.111	74.67 ± 12.31	0.111

DASH scores were measured at week 0 (baseline), week 2, week 4, and week 6, until week 12. There were 2 participants who could not participate until the end of this study. Every week of injection, the participants' DASH score was measured directly. There is a significant decrease in prolotherapy injection patients in each measurement week. There was a significant difference from week 0 to week 6 and at week 12 ($p < 0.05$).

ROM measurements were also carried out directly, from weeks 0 to week 12. ROM measurements were performed to measure the 6 directions of movement of the shoulder joint: flexion, extension, abduction, adduction, internal rotation, and external rotation. There was a significant change in the direction of flexion, extension, and abduction, with statistical measurements showing a significant mean difference in the three directions both from week 0 to week 6 and at week 12 ($p < 0.05$).

The direction of the adduction joints, internal rotation, and external rotation did not show a significant difference in the mean measurements at week 0 and week 6 ($p > 0.05$). However, in statistical measurements, the direction of internal rotation from week 0 to week 12 showed a significant difference ($p < 0.05$).

Measurement of ROM for each direction of shoulder motion pre- and post-injection was carried out at each injection session. On the measurement of ROM, each direction of shoulder motion showed a significant difference in average each week, from week 0 to week 6 ($p <0.05$). Only ROM Extension at week

0 did not show a significant difference in the mean after prolo injection ($p > 0.05$).

TABLE III
ROM AT PRE AND POST INJECTION

Parameter	Baseline	p-value	Week 2	p-value	Week 4	p-value	Week 6	p-value
	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
				ROM				
Pr-I* Flexion	122 ± 22.42	0.0001	136.33 ± 19.31	0.001	153 ± 20.07	0.002	156.33 ± 22.94	0.007
Po-I^ Flexion	147.67 ± 18.88		151.33 ± 20.13		162.33 ± 20.34		162.67 ± 21.7	
Pr-I Extension	35 ± 18.12	0.32	42.33 ± 12.93	0.0001	48 ± 12.5	0.004	53.67 ± 9.53	0.006
Po-I Extension	41 ± 16.16		51.67 ± 7.71		55.33 ± 8.33		57.67 ± 7.98	
Pr-I Abduction	122.67 ± 24.63	0.0001	128 ± 31.2	0.0001	145.67 ± 27.11	0.001	148 ± 33.74	0.002
Po-I Abduction	145.67 ± 16.78		143.667 ± 28.75		159.33 ± 23.89		162.67 ± 19.44	
Pr-I Adduction	47.33 ± 26.65	0.0001	57.33 ± 18.59	0.007	59.67 ± 11.56	0.026	61 ± 8.7	0.042
Po-I Adduction	59 ± 23.46		68.33 ± 17.89		65.33 ± 13.29		66 ± 8.06	
Pr-I Internal Rotation	40.33 ± 18.17	0.001	44 ± 24.28	0.0001	58.67 ± 22.63	0.0001	57 ± 21.61	0.008
Po-I Internal Rotation	53 ± 20		59 ± 20.1		64 ± 22.92		64 ± 20.19	
Pr-I External Rotation	53 ± 26.3	0.002	62.67 ± 20.42	0.007	66 ± 20.1	0.002	69.67 ± 17.47	0.014
Po-I External Rotation	66.67 ± 21.76		71.33 ± 16.41		75.667 ± 15.1		78.33 ± 11.44	

Legend: *Pr-I: Pre-Injection; ^Po-I: Post-Injection

IV. DISCUSSION

This study showed a decrease in the DASH score and an increase in ROM of the shoulder joint after injection using a Dextrose prolotherapy solution. Significant improvement was seen in the ROM pre- and post-injection prolotherapy solution every week. In measuring serum protein MMP-1 and TIMP-1, there was no significant difference before and after injection using prolotherapy solution.

This study showed that injection using prolotherapy glucose solution accompanied by a physiotherapy exercise program effectively reduced complaints due to FS, which was shown in a decrease in the value of the DASH score at each follow-up session. As shown in Table II, there is a significant difference in the DASH score baseline at week 0 and the DASH score at week 6 and week 12.

The measurement of ROM motion of the shoulder joint with limited ROM at week 0 (baseline) shows a significant change in ROM value in the direction of joint movement in flexion, extension, and abduction at week 0 (baseline), week 6, and week 12 follow-up. Significant differences in values are also shown in the direction of motion—internal joint rotation at week 12. Measurements were made by calculating the average direction of motion of the shoulder joint before and after injection of prolotherapy in each session.

Significant changes in shoulder ROM values were also shown in each pre- and post-prolotherapy injection measurement in each session. This significant difference was shown in almost every direction of joint movement: flexion, abduction, adduction, internal rotation, and external rotation, except in the extension direction at week 0 (baseline).

Each participant's blood serum was also taken to assess changes in MMP-1 and TIMP-1 proteins as markers in FS patients. The results showed no significant differences in MMP-1, TIMP-1, and levels of ratio MMP-1/TIMP-1 at week 0 compared to week 6, week 0 compared to week 12, and week 6 compared to week 12. However, although it does not show a significant difference in Table I, it is shown that there is a decrease in the average value of patients after four injection sessions at week 6 compared to serum levels at week 0 (baseline).

Based on our search, very few studies discuss the effectiveness of prolotherapy in patients with FS. However, there are few studies regarding the use of prolotherapy to be effective in pain management in patients with chronic musculoskeletal pain. Nasiri have compared the effectiveness of prolotherapy and corticosteroid injection on shoulder pain [9]. In this study, prolotherapy and corticosteroid injections were equally effective in managing shoulder pain at 3 weeks and 12 weeks of follow-up. Although in conclusion, the use of prolotherapy has fewer side effects than corticosteroid injections.

A systematic review study conducted by Robinson [10] took scientific journals about a non-operative approach to treating rotator cuff disorder and glenohumeral osteoarthritis. In his study, two articles evaluated the use of prolotherapy injections vs. saline as placebo injections. The first study [11] evaluated patients' VAS scores and Ultrasound Shoulder Pathology Rating Scale (USPRS) scores during nine months of follow-up therapy. The results showed a significant difference between prolotherapy injection and saline injection. The second study [12] assessed the Shoulder Pain and Disability Index (SPADI) and VAS score in patients with prolotherapy and saline injection during six weeks of follow-up. There was no significant difference in SPADI scores and VAS scores between prolotherapy and saline injection during the six-week follow-up.

A study that Lubis carried out assessed changes in serum MMP and TIMP levels in 2012 [4], who assessed changes in serum MMP-1, MMP-2, TIMP-1, and TIMP-2 in FS patients who received intensive shoulder training and in patients who did not receive shoulder incentive training. Otherwhile, the pain reduction mechanism in prolotherapy is assumed to occur by its capacity to promote growth-factor mediated tissue healing, the

ability to provide nutrients that necessary for regeneration of tissue that could strengthen the ligament and tendons [13].

Although this study is relatively new in assessing serum levels of MMP-1 and TIMP-1 as a shoulder tissue repairing factor, this is a bold step that we have taken to assess the effectiveness of prolotherapy in FS patients assessed for changes in serum MMP-1 and TIMP-1. The main weakness of this study was the lack of samples that we could collect by the end of the study. The protocol we used was prone to causing patients to drop out or lose contact in the 12 weeks of follow-up.

V. Conclusion

Dextrose prolotherapy injection results give a significant improvement in functional outcome of shoulder joint (DASH score and ROM); however, it did not show significant results in assessing the specific biomarker, MMP, and TIMP in tissue repair. The results obtained may be used as an alternative to the use of injection prolotherapy in FS patients, which has fewer side effects and better effectiveness than the use of corticosteroid injections.

References

[1] Hubbard MJ, Hildebrand BA, Battafarano MM, Battafarano DF. (2018). *Common Soft Tissue Musculoskeletal Pain Disorders*. Prim Care. Jun; 45(2):289-303. (PubMed).

[2] Joseph D. Zuckerman; Andrew Rokito (2011). *Frozen shoulder: a consensus definition, 20(2), 0–325.* doi:10.1016/j.jse.2010.07.008

[3] Cho, C.-H., Song, K.-S., Kim, B.-S., Kim, D. H., & Lho, Y.-M. (2018). *Biological Aspect of Pathophysiology for Frozen Shoulder. BioMed Research International, 2018, 1–8.* doi:10.1155/2018/7274517

[4] Lubis, A. (2013). Matrix metalloproteinase, tissue inhibitor of metalloproteinase and transforming growth factor-beta 1 in frozen shoulder, and their changes as response to intensive stretching and supervised neglect exercise. Journal of orthopaedic science : official journal of the Japanese Orthopaedic Association, 18. doi : 10.1007/s00776-013-0387-0

[5] Kim, Y.-S., Kim, J.-M., Lee, Y.-G., Hong, O.-K., Kwon, H.-S., & Ji, J.-H. (2013). *Intercellular Adhesion Molecule-1 (ICAM-1, CD54) Is Increased in Adhesive Capsulitis.* The Journal of Bone and Joint Surgery-American Volume, 95(4), e18–1– 8. doi:10.2106/jbjs.k.00525

[6] Lee, H. V.; Lee, S. J.; Nazarian, A.; Rodriguez, E. K. (2016). *Adhesive capsulitis of the shoulder: review of pathophysiology and current clinical treatments.* Shoulder & Elbow, 9(2), 75-84. doi:10.1177/1758573216676786.

[7] Xiao RC, DeAngelis JP, Smith CC, Ramappa AJ. (2017). Evaluating Nonoperative Treatments for Adhesive Capsulitis. WINTERJ Surg Orthop Adv. 26(4):193-199.

[8] Zreik NH, Malik RA, Charalambous CP. (2016). *Adhesive capsulitis of the shoulder and diabetes: a metaanalysis of prevalence. Muscles Ligaments Tendons J*; 6:26.

[9] Nasiri A., Jahromi M., Vafei M. A., Parvin R., Fakheri M. S., Sadeghi S. (2021). *Comparison of the Effectiveness of Ultrasound-Guided Prolotherapy in Supraspinatus Tendon with Ultrasound-Guided Corticosteroid Injection of Subacromial Subdeltoid Bursa in Rotator Cuff-Related Shoulder Pain: A Clinical Trial Study*. Advance Biomed Research, 10 (12). doi: 10.4103/abr.abr_181_20

[10] Robinson, D. M., Eng, C., Makovitch, S., Rothenberg, J. B., DeLuca, S., Douglas, S., … Borg-Stein, J. (2021). *Non-operative orthobiologic use for rotator cuff disorders and glenohumeral osteoarthritis: A systematic review. Journal of Back and Musculoskeletal Rehabilitation, 34(1), 17–32.* doi:10.3233/bmr-201844

[11] Bertrand, H., Reeves, K. D., Bennett, C. J., Bicknell, S., & Cheng, A.-L. (2016). *Dextrose Prolotherapy Versus Control Injections in Painful Rotator Cuff Tendinopathy. Archives of Physical Medicine and Rehabilitation, 97(1), 17–25.* doi:10.1016/j.apmr.2015.08.412

[12] Lin, C.-L., Huang, C.-C., & Huang, S.-W. (2019). *Effects of hypertonic dextrose injection in chronic supraspinatus tendinopathy of the shoulder: a randomized placebo-controlled trial.* European Journal of Physical and Rehabilitation Medicine, 55(4*)*. doi:10.23736/s1973-9087.18.05379-0

[13] Waluyo, Y., Budu, Bukhari, A., Adnan, E., Darjanti Haryadi, R., Idris, I., Hamid, F., Usman, A., Johan, M. P., & Zainuddin, A. A. (2021). Changes in levels of cartilage oligomeric proteinase and urinary C-terminal telopeptide of type II collagen in subjects with knee osteoarthritis after dextrose prolotherapy: A randomized controlled trial. *Journal of Rehabilitation Medicine*, *53*(5). https://doi.org/10.2340/16501977-2835