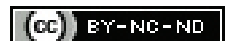


Effectiveness of Ultrasound-guided Platelet-rich Plasma Injection vs Pulsed Ultrasound Therapy on Improving Pain and Function in Athletes with Medial Collateral Ligament Injury of Knee: A Randomised Controlled Trial

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ABSTRACT

Introduction: The Medial Collateral Ligament (MCL) is the most frequently injured ligament in the knee. Platelet-rich Plasma (PRP) is an autologous concentration of platelets prepared as an injection to augment the healing process.

Aim: To determine the effectiveness of Ultrasound (USG)-guided PRP injection in comparison with pulsed ultrasound therapy in improving pain and function in athletes with MCL injury.

Materials and Methods: A randomised control trial was done among athletes with partial tears of MCL who visited the Department of Sports Medicine, Regional Institute of Medical Sciences, Imphal, Manipur, India from October 2020 to September 2022. Patients with Magnetic Resonance Imaging (MRI)-diagnosed partial tears of MCL (N=32) were randomised into PRP injection (n=16) and pulsed ultrasound therapy (n=16) groups. The outcomes were compared using the Visual Analogue Scale (VAS) for pain and Lysholm score for function at baseline, 2nd, 6th, and 12th weeks, respectively. Analysis was done using International Business Machines-Statistical Package for the

Social Sciences (IBM-SPSS) version 21.0. For comparison between the groups, independent t-test was used. A p-value <0.05 was considered statistically significant.

Results: Baseline characteristics were not statistically significant. There was a statistically significant improvement seen in the intragroup comparison from baseline to the 2nd, 6th, and 12th weeks in both VAS (p<0.001*) and Lysholm score (p<0.001) in both groups. There were statistically significant differences in mean changes of VAS and Lysholm score between the two groups from baseline to the 2nd week (VAS, p=0.008*); (Lysholm score, p=0.003) and 6th week (VAS, p<0.001*); (Lysholm score, p=0.009) follow-ups respectively, with the PRP group having more improvement. However, there was no significant difference at 12th week (VAS, p=0.088); (Lysholm score, p=0.072) weeks.

Conclusion: Given the better results, it is suggested that ultrasound-guided PRP injection may be used as the preferred method of treatment in the management of MCL injuries to return to sports as early as possible.

Keywords: Athletes, Knee function, Knee ligament, Lysholm, Visual analogue score

INTRODUCTION

The MCL stands as the most frequently injured ligament within the knee, located prominently on its medial aspect [1-4]. Comprising both superficial and deep components, the MCL provides crucial static stabilisation against valgus stress [1]. MCL injury manifests as a traumatic distraction affecting both the superficial (sMCL) and deep (dMCL) components, resulting in diminished participation in training or match play [2]. Given the vulnerability of the lateral knee during sports activities, MCL injuries prevail as the most common ligamentous knee injury [1]. While a valgus force on a flexed knee represents the typical mechanism, severe MCL injuries may involve additional forces, particularly in high-energy trauma or complex knee injury patterns. These injuries may arise in isolation or concomitantly with multi-ligamentous, meniscal, or other associated knee pathologies [3].

The MCL sustains injury in approximately 42% of knee ligament injuries, with isolated MCL injuries accounting for 29% of knee ligament injuries alone [5]. These injuries are frequently encountered in athletic populations, particularly in contact sports such as American football, soccer, wrestling, hockey, and rugby. Evaluation of MCL integrity involves valgus stress testing of the knee, conducted both at full extension and 30° of flexion. At full extension, the test assesses the integrity of the superficial and deep MCL, as well as the Anterior Cruciate Ligament (ACL) and PCL. Conversely, valgus

stress examination at 30° of flexion specifically isolates the MCL. The gold standard for diagnosing MCL injuries is T2-weighted MRI imaging [6]. Clinically, MCL injuries are graded [7]. Grade I injuries typically present with local tenderness over the MCL, without significant swelling, and a valgus stress test at 30° of flexion causes pain without increased laxity, indicating intact ligament integrity. In Grade II injuries, there is marked tenderness, often accompanied by localised swelling. Valgus stress at 30° of flexion induces pain and demonstrates some laxity with a distinct endpoint, while the knee remains stable at full extension, indicating compromised but intact ligament integrity throughout its length. Grade III injuries are characterised by tenderness over the ligament and gross laxity upon valgus stress at 30° of flexion, without a distinct endpoint. Additionally, minor valgus instability may be evident at full extension [7].

Grade 1 and 2 MCL injuries are typically treated conservatively through early rehabilitation, including range of motion exercises and progressive strength training [1,8]. Similarly, isolated grade 3 tears follow a non surgical approach. However, failure of conservative treatment can lead to persistent medial instability, anterior cruciate ligament dysfunction, weakness, and osteoarthritis [9]. Both amateur and professional athletes aim for an early return to play. Various emerging therapies like prolotherapy, PRP injection, stem cells, and others are under exploration to expedite healing [6].

The PRP is a promising orthobiologic substance for minimally invasive knee lesion treatment. It enhances healing by releasing cytokines and Growth Factors (GF) such as interleukin 1 β , interleukin 8, Tumour Necrosis Factor-alpha (TNF- α), Platelet-derived Growth Factor (PDGF), PDEGF, Transforming Growth Factor- β 1 (TGF- β 1), Insulin-like Growth Factor (IGF-1), Fibroblast Growth Factor-2 (FGF-2), Hepatocyte Growth Factor (HGF), and Vascular Endothelial Growth Factor (VEGF) from alpha granules. These factors stimulate cell proliferation, migration, and differentiation, influencing the immune system, inflammation, and angiogenesis [10]. The healing process begins with platelet aggregation and clot formation, forming a scaffold that supports cell growth and differentiation [11].

Research on Low-intensity Therapeutic Ultrasound (LITUS) suggests its positive biomechanical effects on soft tissue recovery, enhancing tissue repair processes [12,13]. Ultrasound stimulates mast cell degranulation, histamine release, and macrophage responsiveness while increasing protein synthesis and nitric oxide production [14]. Although most effective during the inflammatory phase, its benefits extend throughout the repair phases. LITUS doesn't alter the repair process but enhances it, leading to a more efficient resolution of clinical issues [13]. Hence, using low-intensity pulsed ultrasound post-ligament injury may expedite athletes' return to activity after MCL injury [15].

There are very less studies determining the effectiveness of PRP injection in the treatment of MCL injury [11,16-18]. The greatest limiting factor for PRP is the lack of standardisation. More research needs to be conducted to understand how leukocyte inclusion, activation, and platelet concentration affect therapeutic efficacy [19]. The treatment of acute MCL lesions with PRP is a promising therapeutic option which needs further explored with good quality Randomised Controlled Trials (RCTs) [16]. Therefore, a randomised controlled study was done to determine the effectiveness of ultrasound-guided PRP injection which has been compared with pulsed ultrasound therapy in the treatment of MCL knee injury and to bring the athletes back to sports in the shortest possible time.

MATERIALS AND METHODS

An RCT was done among athletes with partial MCL injury who visited the Department of Sports Medicine, Regional Institute of Medical Sciences, Imphal from October 2020 to September 2022. The approval of the Research Ethics Board, Regional Institute of Medical Sciences, Imphal, was taken for the present clinical study (REB No. A/206/REB-Comm(SP)/RIMS/2015/748/90/2020). The study was registered in the Clinical Trials Registry of India, and the registration number was CTRI/2021/04/032936. Patients with medial knee pain following injury were clinically examined and sent for an MRI. Patients with MRI-diagnosed grade 1 and 2 MCL injuries (n=32) were randomised into PRP injection plus rehabilitation (n=16) and pulsed ultrasound therapy plus rehabilitation (n=16) groups.

Patients were informed about the nature of the study, and willing participants were asked to sign the informed consent form.

Inclusion criteria: Patients with MRI-confirmed grades 1 and 2 MCL injuries of the knee among the age group 18-35 years within two weeks of injury.

Exclusion criteria: Patients with any associated ligament and meniscal injuries other than MCL confirmed by MRI or any previous knee surgery were excluded from the study. Those with knee instability due to hypermobility syndrome, local infection around the knee, uncontrolled systemic diseases, thrombocytopenia (platelet count less than 150,000 per microlitre), and those who received local corticosteroid injection within six weeks were also excluded from the present study.

Sample size calculation: A sample size of 32 was calculated using the formula:

$$N=(Z\alpha+Z\beta)^2(S1^2+S2^2)/(m1+m2)^2$$

Where,

$Z\alpha=2.57$ at 1% level of significance

$Z\beta=1.96$ at 95% power

Taking into consideration the study conducted by Sharaki F et al., in 2019 [11].

The findings were:

$m1$ (mean VAS score in the control group)=2.43

$m2$ (mean VAS score in the intervention group)=1.30

$s1$ (standard deviation of VAS score in the control group)=0.507

$s2$ (standard deviation of intervention group)=0.765

Sample was calculated accordingly as:

$$N=\frac{(2.57+1.96)^2 \times (0.507^2 + 0.765^2)}{(2.43-1.30)^2}$$

=13.6

≈ 14 (round off)

Hence, 14 patients needed to be studied per group, giving a total of 28 patients. However, taking into consideration a dropout rate of 10%, the final calculated sample comes to 15.4 (≈ 16) patients per group, giving a total sample size of 32 patients.

Outcome measures: Knee pain was measured by VAS and knee function was measured by Lysholm Knee Scoring Scale [11].

Study Procedure

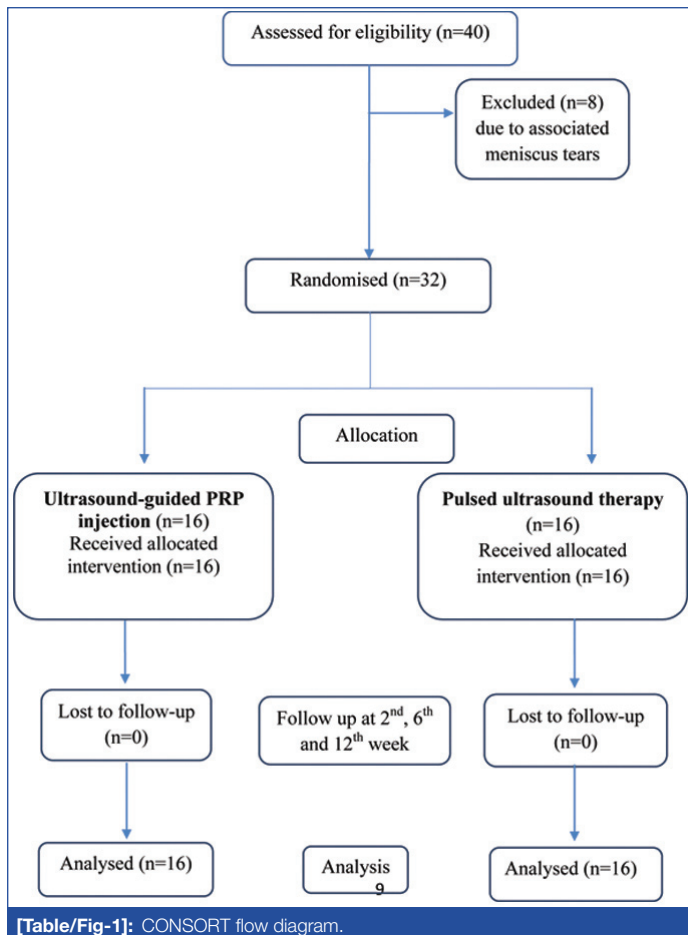
Patients who met inclusion and exclusion criteria and gave consent for participation in the study were randomised (n=32) into PRP injection plus rehabilitation (n=16) and pulsed ultrasound therapy plus rehabilitation (n=16) groups by block randomisation technique. A block size of four was used. Possible treatment allocations within each block were: (i) AABB; (ii) BBAA; (iii) ABAB; (iv) BABA; (v) ABBA; (vi) BAAB. Using a computer-generated table, a list of 8 blocks was prepared to reach a sample size of 32.

Darting was done to select a block by using a pen after closing the eyes. For each selected block, there was a sequence of treatment options. The sequence of treatment options in each block was put in an opaque envelope and sealed. The corresponding envelope was labelled 1, 2, 3, 4... up to 32 according to the appearance of treatment allocation in each selected block. The sealed envelope with label 1 was opened only when we had the first eligible patient and the treatment was allocated. Single blinding was done in which the assessor was blinded.

After taking informed consent, age, gender, type of sports, duration, MRI grading, BMI, side of affection were recorded, and all the subjects were evaluated for Lysholm score and VAS at baseline before starting any intervention. After starting the interventions, at the end of the 2nd week, 6th week, and 12th week, patients were evaluated with Lysholm score and VAS. [Table/Fig-1] shows the Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

PRP injection plus rehabilitation (Study group): The PRP was prepared using the Double spin method [20]: 18 mL of whole blood was obtained by venipuncture in Acid Citrate Dextrose (ACD) tubes. Centrifugation was done using a 'soft' spin at 2400 rpm for 10 minutes; the supernatant plasma containing platelets was collected and centrifuged again at a hard spin, i.e., 3600 rpm for 15 minutes to obtain a platelet concentrate. The lower 1/3rd is PRP, and the upper 2/3rd is Platelet-poor Plasma (PPP). 2 mL of PRP was procured by removing the PPP.

The patient was made to lie comfortably in a supine position with the knee flexed to 20 degrees and resting on a pillow. The hip may be slightly externally rotated. The skin of the affected area was prepared aseptically and draped by a sterile green sheet. The transducer of the ultrasound was placed in the anatomical sagittal plane over the MCL to localise the site of the injury [21]. A 5 mL



syringe with a 21 Gauge needle size was introduced in the long axis of the transducer, and 2 mL of PRP was injected slowly at the site of the injury. The needle was removed, and local haemostasis was achieved by applying pressure over the injection site, and sterile dressing was done.

Pulsed ultrasound therapy plus rehabilitation (Control group):

The patient was made to lie comfortably in a supine position with the knee flexed to 20 degrees and resting on a pillow, exposing the affected area. The hip may be slightly externally rotated. Pulsed ultrasound with a 20% duty cycle, frequency of 3 MHz was used at an intensity of 0.5 W/cm² for 10 minutes, five times a week for two weeks. Ultrasound gel was applied to the transducer of the ultrasound probe and moved in a slow circular motion around the affected area. The ultrasonic machine, SONOPULS 590 Freq. 1-3 MHz 10W, Enraf-Nonius made in Holland, was used.

Both groups followed a functional rehabilitation program, including range of motion exercises of the knee, progressive strengthening of the quadriceps, hamstring, calf, hip abductor and extensor muscles, and neuromuscular proprioceptive balance training [7].

Follow-up was done at the end of the 2nd, 6th, and 12th weeks for both the intervention group and the control group using VAS for pain and Lysholm knee score for functional status.

STATISTICAL ANALYSIS

Analysis was done using IBM-SPSS version 21 Armonk, NY: IBM Corp. For descriptive statistics, standard deviation, mean, frequency, and percentage were used. Characteristics of the study participants for categorical variables between the study and control groups were analysed by using Chi-square test. For comparison between the groups (intervention group and control group) of outcome variables, an independent t-test was used. For within-group comparison (baseline and follow-up), repeated-measures Analysis of Variance (ANOVA) was used. A p-value <0.05 was considered statistically significant.

RESULTS

The baseline characteristics of the patients in the control and intervention groups were not statistically significant [Table/Fig-2]. Most of athletes were in the age group of 18-25 years, with 19 (56%) in both the study and control groups. The mean age of the study participants was 21.5 years. Among them, females were affected more than males (62.5% in the study group and 56.3% in the control group). Soccer was the most common sports discipline where MCL injured in the present study (50.0% in the study group and 50.0% in the control group). Most of the patients in the present study had MCL grade 2 injuries (56.3% in the study group and 62.5% in the control group). The majority of patients had right-sided involvement (62.5% in the study group and 68.8% in the control group) [Table/Fig-2].

Variables	Groups		p-value
	PRP injection (n) (%)	Pulsed ultrasound therapy (n) (%)	
Age (years)			
18-25	9 (56.3%)	9 (56.3%)	1.000
26-35	7 (43.8%)	7 (43.8%)	
Gender			
Male	6 (37.5%)	7 (43.8%)	0.500
Female	10 (62.5%)	9 (56.3%)	
Type of sports			
Soccer	8 (50.0%)	8 (50.0%)	1.00
Judo	3 (18.8%)	3 (18.8%)	
Badminton	2 (12.5%)	2 (12.5%)	
Taekwondo	1 (6.3%)	1 (6.3%)	
Wushu	1 (6.3%)	1 (6.3%)	
Basketball	1 (6.3%)	1 (6.3%)	
Duration			
0-<1 week	9 (56.3%)	8 (50.0%)	0.500
1-2 weeks	7 (43.8%)	8 (50.0%)	
MRI grading			
Grade 1	7 (43.8%)	6 (37.5%)	0.719
Grade 2	9 (56.3%)	10 (62.5%)	
BMI (kg/m²)			
18.5-<20.5	3 (18.8%)	3 (18.8%)	0.924
20.5-<22.5	5 (31.3%)	6 (37.5%)	
22.5-24.5	8 (50.0%)	7 (43.8%)	
Side of affection			
Right	10 (62.5%)	11 (68.8%)	0.710
Left	6 (37.5%)	5 (31.3%)	

[Table/Fig-2]: Comparisons of background characteristics between the PRP injection (study) and pulsed ultrasound therapy (control) group (N=32).

Chi-square test; p-value <0.05 is taken as significant

The baseline characteristics for both VAS and Lysholm scores are shown in [Table/Fig-3], which showed that there was no statistical significance between the two groups.

Outcome measures	PRP injection (n=16) (Mean±SD)	Pulsed ultrasound therapy (n=16) (Mean±SD)	p-value
VAS	8.73±0.54	8.70±0.56	0.850
Lysholm score	33.44±5.17	32.31±5.14	0.542

[Table/Fig-3]: Comparisons of baseline VAS and Lysholm scores between the PRP and pulsed ultrasound therapy groups (N=32).

Independent t-test; p-value <0.05 is taken as significant

The mean VAS and Lysholm scores of both the study and control groups at baseline and post-intervention at the 2nd week, 6th week, and 12th week is displayed in [Table/Fig-4]. It shows significant improvement within the group in both outcome measures at all time points in both groups.

Parameters	Group	Baseline	2 nd week	6 th week	12 th week	p-value
VAS	PRP injection	8.73±0.54	6.63±0.39	4.83±0.30	2.81±0.69	<0.001**
	Pulsed ultrasound therapy	8.70±0.56	7.19±0.43	5.60±0.45	3.32±0.47	<0.001**
Lysholm score	PRP injection	33.44±5.17	52.94±4.83	65.69±3.11	88.38±2.06	<0.001**
	Pulsed ultrasound therapy	32.31±5.14	46.13±2.75	58.75±3.02	81.50±3.07	<0.001**

[Table/Fig-4]: Intragroup comparisons of VAS and Lysholm scores from baseline to 2nd week, 6th week and 12th week. Repeated measures ANOVA; p-value <0.05 is taken as significant

The mean changes from the baseline of VAS score in both groups at different follow-ups and a comparison of the same between the two groups is shown in [Table/Fig-5]. It reveals a statistically significant difference between the two groups at the 2nd week and 6th week post-intervention, with the PRP injection group having more improvement. At the 12th week post-intervention, there was no statistically significant difference in mean changes from the baseline of VAS score between the two groups.

VAS score	Mean changes from base line		Mean difference	95% CI of difference		p-value
	PRP injection (n=16) (Mean±SD)	Pulsed ultrasound therapy (n=16) (Mean±SD)		Lower	Upper	
2 nd week	-2.10±0.68	-1.50±0.49	0.593	0.165	1.022	0.008*
6 th week	-3.90±0.65	-3.09±0.63	0.806	0.339	1.272	<0.001*
12 th week	-5.91±0.88	-5.37±0.85	0.543	-0.085	1.172	0.088

[Table/Fig-5]: Intergroup comparison of mean changes of VAS from baseline to 2nd week, 6th week and 12th week follow-up. Independent t-test; p-value <0.05 is taken as significant

The mean changes from the baseline of Lysholm score in both groups at different follow-ups and a comparison of the same between the two groups is shown in [Table/Fig-6]. It shows that there was a statistically significant improvement in the Lysholm score at the 2nd week and 6th week post-intervention in the PRP plus rehabilitation group compared to the pulsed ultrasound therapy plus rehabilitation group. At the 12th week, there was no significant difference in the improvement of the Lysholm score between the two groups.

Lysholm score	Mean changes from baseline		Mean difference	95% CI of difference		p-value
	PRP injection (n=16) (Mean±SD)	Pulsed ultrasound therapy (n=16) (Mean±SD)		Lower	Upper	
2 nd week	19.50±5.08	13.81±4.98	-5.687	-9.322	-2.052	0.003*
6 th week	32.25±5.53	26.43±6.17	-5.812	-10.045	-1.579	0.009*
12 th week	52.56±5.99	48.43±6.49	-5.75	-10.040	-1.459	0.072

[Table/Fig-6]: Intergroup comparison of mean changes of Lysholm scores from baseline to 2 weeks, 6 weeks and 12 weeks follow-up (N=32). Independent t-test; p-value <0.05 is taken as significant

DISCUSSION

The healing process of PRP injection commences with platelet aggregation and clot formation, establishing a scaffold that serves as a temporary matrix for cellular growth and differentiation. Platelets play an active role by releasing presynthesised growth factors and synthesising additional growth factors for several days throughout their lifespan [11]. Numerous biological and non biological therapies have been studied either individually or in combination to accelerate the healing process and reduce recovery time. PRP may offer promising results in treating these injuries due to its ease of use, affordability, technical accessibility for production, potential advantages, likely absence of contraindications, and minimal adverse effects [22].

In the present study, the authors compared PRP injections guided by ultrasound with pulsed ultrasound therapy for treating torn MCL,

alongside a rehabilitation program. Both groups showed significant pain and function improvement over time. There was significant improvement in the mean scores of all the outcome measures, i.e., VAS and Lysholm scores in both groups at the 2nd, 6th, and 12th weeks follow-up period (p<0.05). In a study conducted by Sharaki F et al., a similar finding was reported, showing significant improvement in the VAS score from the first visit to 4, 8, and 12 weeks (p<0.001) [11]. In a retrospective study conducted by Zou G et al., significant improvement in VAS scores was observed at the 1st week, 1st month, 3rd month, and 6th-month follow-up evaluations [18]. Algawwam HG et al., conducted a retrospective study where 27 patients (87.0%) received only one session of ultrasound-guided PRP injections and stated also that a single injection of PRP had statistically significant decrease which assisted in faster rehabilitation progress, shorter time spent out of play, and less time without exercise, which is vital to professional athletes [19]. When a comparison was done between the two groups, more improvement in VAS was noticed in the study group in all the follow-ups, i.e., 2nd week, 6th week, and 12th week. Significant improvement was seen in the study group in VAS at the 2nd week (p=0.008) and 6th week (p=0.001) follow-ups. In a study conducted by Sharaki F et al., significant improvement in the VAS score was seen in the study group, i.e., the PRP group, in the 4th-week follow-up [11]. You CK et al., discovered that administering PRP injections three times under ultrasound guidance reduced pain and instability and enhanced the healing of ACL tears post-intervention [23]. Zhang J et al., observed that the group receiving two PRP injections showed significantly better clinical outcomes and improved quality of the ATFL in the short-term follow-up [24]. Mill FB IV et al., found PRP therapy to be promising for Types I and II Ulnar collateral ligament tears, with Type III showing high success rates but limited data [25].

The Lysholm scores also showed improvement at all follow-ups in both groups. This is because, as the pain improved, patients were able to perform activities and progress functional rehabilitation programs without any limitations, and thus they had improvement in their function and could achieve faster return to sports activities. There was a significant improvement in Lysholm score in both groups separately (p<0.05). In a study conducted by Sharaki F et al., Lysholm scores at 4, 8, and 12 weeks compared to the first visit had statistically significant improvement (p<0.001) in both groups, which is similar to the present study [11]. When a comparison was done between the two groups, more improvement in Lysholm was noticed in the study group at all follow-ups, i.e., 2nd week, 6th week, and 12th week. In a study conducted by Sharaki F et al., Lysholm scores of the groups at the first visit, 4th, 8th, and 12th week visits had no statistically significant difference (p=0.363) [11]. Laimujam SD et al., discovered that combining PRP injection with rehabilitation accelerates recovery, allowing for a quicker return to play by expediting rehabilitation progress [20].

Ultrasound-guided PRP injection, being a minimally invasive procedure, is not a first-line therapy for the management of partial MCL tear. However, as it is highly effective, it may be considered as an adjunct to the standard functional rehabilitation program for patients with partial MCL tear, enabling them to return to their sports activities as earliest as possible.

Many patients who received PRP via single-spin methods experienced post-injection flare-ups. These flare-ups included pain, redness,

and swelling at the injected knee. This reaction could be attributed to the fact that single-spin PRP yields a leukocyte-rich formulation. The high concentration of leukocytes in PRP can elevate levels of catabolic and pro-inflammatory signaling molecules, such as MMPs and IL-1 β [26]. Saqlain N et al., found that the double centrifugation method resulted in a higher platelet quantity and yield with less contamination by red and white blood cells than did the single centrifugation method for PRP preparation [27]. In this study, double spin PRP was given, and there were no serious complications in both group after the procedure. There was mild swelling and mild pain at the injection site that subsided with the application of ice. Patients were advised to rest for 2-3 days and gradually started a functional rehabilitation program as tolerated. PRP injection under ultrasound guidance can be proposed as an effective treatment option for the conservative management of partial MCL tear to improve pain and, most importantly, to improve function, allowing athletes to return to their sports activities as earliest as possible.

Limitation(s)

The study could have been more reproducible if done with a bigger sample size and longer follow-up periods.

CONCLUSION(S)

Ultrasound-guided PRP injection is a safe, minimally invasive, and effective treatment for partial MCL tears. Both PRP injection and pulsed ultrasound therapy improved pain and function up to the 12th week. PRP injection showed greater pain relief and functional improvement, suggesting it as the preferred treatment for managing pain, improving function, and facilitating a faster return to sports activities in MCL tear patients. However, further studies with larger sample sizes and longer follow-up periods are recommended to support these findings.

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