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EFFECTS OF BLOOD FLOW RESTRICTION WITH AND WITHOUT TOTAL MOTION RELEASE ON RANGE OF MOTION IN POST-OPERATIVE ACL INJURY: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Background and Objectives: *One key component of the rehabilitation process following anterior cruciate ligament (ACL) reconstruction is restoration of knee range of motion (ROM), and maintaining a functional range of motion without excessive flexion or terminal extension deficit is important for promoting gait normalization and functional recovery. This RTC compared the influence of Blood Flow Restriction + Total Motion Release (BFR+TMR) on knee ROM in the rehabilitation of ACL injury.*

Methods: *Forty-four patients who underwent ACL reconstruction surgery were randomly assigned to the BFR group (n = 22) and BFR + TMR group (n = 22). ROM was measured at baseline, 6 weeks, 12 weeks and 6 months for knee flexion of injured limb, knee extension deficit of injured limb, knee flexion of non-injured limb and knee extension deviation of non-injured limb. Two-way mixed ANOVA with time (the within-subjects factor) and group (the between-subjects factor) was used to analyze data.*

Results: *All outcomes of the ROM showed significant time effects and Time × Group interactions. Injured-limb flexion improved from 63.05 ± 5.00 degrees to 93.86 ± 5.48 degrees in the BFR group and from 64.27 ± 5.36 degrees to 127.91 ± 4.47 degrees in the BFR+TMR group, with a significant Time × Group interaction, $F(3,126) = 355.17$, $p < .001$, partial eta squared = .894. There was also a significant interaction for injured-limb extension deficit, with the BFR group showing improvement of -0.82 ± 0.73 degrees and BFR+TMR group showing improvement of -0.14 ± 0.35 degrees, $F(3,126) = 7.93$, $p < .001$, partial eta squared = .159. There was also a significant interaction effect between the non-injured limb flexion and extension.*

Conclusion: *In the framework of ACL injury rehabilitation, the use of Total Motion Release in addition to BFR yielded significantly higher and quicker ROM recovery compared to BFR alone. This combination of protocols can clinically be beneficial in enhancing knee flexion, terminal extension and bilateral movement symmetry.*

Clinical Trial Number: *NCT07589309*

Keywords: *Anterior cruciate ligament; Blood Flow Restriction; Total Motion Release; Range of Motion; Knee flexion; Knee extension*

1. INTRODUCTION

Anterior cruciate ligament (ACL) is not an uncommon injury in people who are active, however quadriceps muscle weakness, impaired balance, and functional limitations are often evident for months and even years after surgery, before safe return to sport which increases long-term osteoarthritis risk.[1]

ACL injuries are among the most common knee injuries in athletes. They are particularly prevalent in sports that involve pivoting movements, such as cricket, football, basketball, netball, soccer, handball, gymnastics, and downhill skiing. These injuries can vary in severity, ranging from minor sprains or partial tears to complete ruptures of the ligament.[2]

ACL injuries may occur through both contact and non-contact mechanisms, although non-contact injuries are more frequent, typically involving valgus stress combined with internal rotation when the foot is planted.[3] Evidence also indicates that risk of ACL injury is slightly higher in female instead of male, with an observed injury ratio of approximately 1.38 times greater in females participating in similar sports activities.[4]

Globally, ACL injury incidence in the general population is estimated at approximately 0.03% per year (around 1 in 3,500 individuals or 68 per 100,000 population annually).[5] In Pakistan, ACL injury is a commonly reported knee ligament injury in orthopedic and sports trauma clinics, with hospital-based studies showing a high proportion among knee injury cases in young active individuals. It predominantly affects males aged 15–35 years, especially athletes, with most cases resulting from non-contact sports mechanisms such as pivoting and landing. [6]

Anterior cruciate ligament reconstruction (ACLR) is widely regarded as a surgical procedure for restoring mechanical stability to the knee joint. However, despite improvements in surgical techniques, effective postoperative rehabilitation remains essential for achieving optimal functional outcomes. Research indicates that ACLR is often associated with marked muscle atrophy, especially in the quadriceps, along with persistent deficits in neuromuscular control and strength. [7]

Blood Flow Restriction (BFR) training is a new and increasingly utilized rehabilitation strategy in musculoskeletal and sports physiotherapy. It involves the application of controlled external pressure to a limb using pneumatic cuffs or elastic bands, with the aim of partially restricting arterial inflow while occluding venous return during exercise. This unique physiological environment allows individuals to achieve significant improvements in hypertrophy and muscle strength while performing low-load resistance exercise, typically at 20–30% of one-repetition maximum (1RM), which would otherwise be insufficient to induce such adaptations. [8]

Current evidence from systematic reviews and meta-analyses indicates that Blood Flow Restriction can lead to meaningful improvements in muscle strength, muscle mass, and patient-reported outcomes after ACLR.[9]

BFR training enables patients to perform exercise at low external loads while achieving strength and hypertrophy adaptations compare to high load training is an attractive option when high joint loads are contraindicated early after ACLR. Recent randomised trials and meta-analyses investigating cohorts of patients with ACLR have reported improvements following BFR in pain, isokinetic strength and patient-reported outcomes (e.g. IKDC), but this has not been the case for muscle volume and mid-term function, which has been mixed across studies.[10]

In recent years, BFR training has gained substantial attention in orthopedic rehabilitation, especially in conditions such as anterior cruciate ligament (ACL) reconstruction, where rapid restoration of muscle strength and function is essential for optimal recovery.[11] Its ability to minimize muscle atrophy and accelerate strength gains without imposing excessive joint stress positions BFR as a promising adjunct to conventional rehabilitation protocols.[12]

Total Motion Release (TMR) is an emerging therapeutic approach that focuses on restoring movement symmetry and neuromuscular control through the concept of regional interdependence. Unlike conventional therapies that primarily target the injured site, TMR utilizes movements performed on the non-affected or less painful side to produce beneficial effects on the affected side through central nervous system mechanisms.[13]

Total Motion Release (TMR) utilizes asymmetry: Treatment of the non-painful, or contralateral, side to elicit rapid and overall changes in movement, ROM and pain-possibly through neurophysiologic cross-education and regional interdependence mechanisms.[14]

The theoretical foundation of Total Motion Release (TMR) is closely linked to the principle of cross-education, where training one limb can lead to improvements in strength and motor control in the opposite, untrained limb. This concept is especially relevant in Anterior Cruciate Ligament Injury rehabilitation, where patients often present with bilateral neuromuscular deficits and altered movement patterns due to compensation. By promoting better motor control and addressing asymmetries, TMR has the potential to improve functional performance and may also help lower the risk of reinjury.[15]

Although Total Motion Release (TMR) is being adopted more widely in clinical settings, there is still a small amount of high-quality randomized controlled trials supporting its effectiveness. Much of the existing evidence comes from clinical experience, case reports, and rehabilitation models rather than large-scale experimental studies. This represents an important gap in the literature, especially in orthopaedic populations such as patients undergoing Anterior Cruciate Ligament Injury reconstruction.

Even with limited direct evidence, TMR is consistent with well-established rehabilitation principles, including motor learning, neuroplasticity, and cross-education. These theoretical foundations provide support for its potential clinical value in improving movement quality and functional recovery.

Total Motion Release (TMR) is a movement-focused rehabilitation method that targets asymmetries through a global assessment of the kinetic chain. It emphasizes using pain-free movement patterns, often involving the opposite limb, to influence mobility and control in the affected side. This makes it especially relevant in postoperative cases such as Anterior Cruciate Ligament Injury reconstruction, where early rehabilitation is often limited by healing constraints and load restrictions.

BFR training has been found to stimulate marked muscle strength and hypertrophy with low load resistance exercise, and is, therefore, a better choice, especially in the early postoperative period when high intensity exercise is contraindicated. Nevertheless, BFR mainly refers to muscular adaptations and lacks direct attention to the movement symmetry and neuromuscular controls deficits. [16]

BFR + TMR could be a complementary rehabilitation plan. The local muscular adaptability possibilities offered by BFR are safe and applicable to low-load exercise, while the global neuromuscular mechanisms offered by TMR are applicable to improve movement quality and symmetry. The purpose of this study was to determine the effects of BFR alone versus BFR and TMR on knee flexion and extension ROM in patients who have previously suffered from an ACL injury.

2. Methods

2.1 Study Design

A randomized controlled trial (NCT07589309) was performed to assess the effects of BFR training alone and BFR combined with TMR (total motion release) on knee ROM in post operative ACL rehabilitation.

2.2 Setting and Duration

Study was conducted at Department of Rehabilitation Sciences, Sir Ganga Ram Hospital, Lahore. Supervised intervention was for 12 weeks; outcomes were measured at baseline, 6 weeks, 12 weeks, and 6 months.

2.3 Participants and Randomization

44 post-operative ACL reconstruction patients were randomly assigned to two equal groups: BFR only (n=22) and BFR+TMR (n=22). After the baseline assessment, eligible participants were randomized by a sealed-envelope method.

2.4 Inclusion Criteria

- Age 16-45 years.
- Male and female patients.

- Primary unilateral ACL reconstruction using hamstring or patellar tendon autograft.
- Two to four weeks post-operative and cleared for supervised rehabilitation.
- Willing and able to provide written informed consent.

2.5 Exclusion Criteria

- Revision ACL surgery or multi-ligament injury.
- Concomitant fracture or meniscal repair requiring restricted rehabilitation.
- History of deep vein thrombosis, vascular disease, uncontrolled hypertension, or cardiovascular disease.
- Neurological disorders affecting lower-limb movement or control.
- Contraindications to BFR, including pregnancy or clotting disorders.

2.6 Intervention Protocol

Both groups received supervised rehabilitation three times per week. Supportive rehabilitation included warm-up, light mobility exercises, stretching and a home exercise programme to maintain continuity between supervised sessions.

Group A: BFR + TMR. The combined group was trained with TMR prior to BFR training. The use of TMR to treat movement asymmetry and encourage pain free movement. Active movement tests including trunk rotation and side bending, hip movement patterns, mini squats and weight shifts were used to measure movement asymmetry. The exercises were primarily performed on the contralateral or less restricted side and involved trunk rotation, side bending at the trunk, hip shift, knee extension in sitting position on the non-painful side and single-leg stance on the non-painful side. All exercises performed for 3 sets of 10 reps at short rest intervals followed by reassessment and progression based on movement symmetry, range of motion and decreased discomfort.

Following TMR, BFR training was given by applying an inflatable cuff to the proximal thigh of the affected limb. The 60-80% of LOP was individualized as occlusion pressure. The exercises performed were 30-15-15-15 with 30-45 seconds of rest between sets using low load (20-30% of 1RM) resistance, and included mini squats, knee extensions, and straight leg raises.

Group B: BFR Only. The BFR-only group was given BFR strengthening exercises without the addition of TMR. The proximal thigh of the affected limb was placed under a cuff pressure of 60-80% limb occlusion pressure. With the same low-load repetition structure, exercises involved were knee extensions, straight leg raises, and mini squats.

2.7 Outcome Measures

ROM of the knee was measured in degrees at baseline, 6 weeks, 12 weeks and 6 months. The main outcomes of the ROM were the injured-limb knee flexion, injured-limb knee extension deficit, non-injured limb knee flexion, and non-injured limb knee extension deviation from terminal extension. The positive joint angles were assigned to flexion, while the negative angles were assigned to the deficits in extension or deviations from terminal extension (0 degrees). Same standard measurement procedure applied at each time point.

2.8 Ethical Considerations

Ethical approval (GIU/REC/26-30) was obtained from the Research Ethical Committee of Green International University, Lahore, Pakistan. Written informed consent was obtained from all participants before data collection.

2.9 Statistical Analysis

IBM SPSS Statistics, Version 26.0 software was used for data analysis. Continuous variables were reported as mean and standard deviation and categorical variables were reported as frequencies. Independent-samples t-test was used to examine baseline equivalence between groups on age and BMI; Pearson chi-square test was used to examine gender distribution baseline equivalence between groups. Two-way mixed analysis of variance (ANOVA) was used to analyze the outcome of the ROM. Time was the within-subjects factor, and treatment group was the between-subjects factor. Main effects for the time and Time × Group interaction effects were reported using F-statistics, p-values and partial eta squared. P value of less than .05 was considered statistically significant.

3. Results

3.1 Participant Flow and Baseline Characteristics

During recruitment, 57 post-operative ACL cases were screened for eligibility. Thirteen participants were excluded, and 44 eligible participants were randomized into two groups of 22 participants each. Two participants from the BFR group and one participant from the BFR+TMR group were lost to follow-up for personal reasons; randomized participants were retained in the analysis according to the available-data approach.

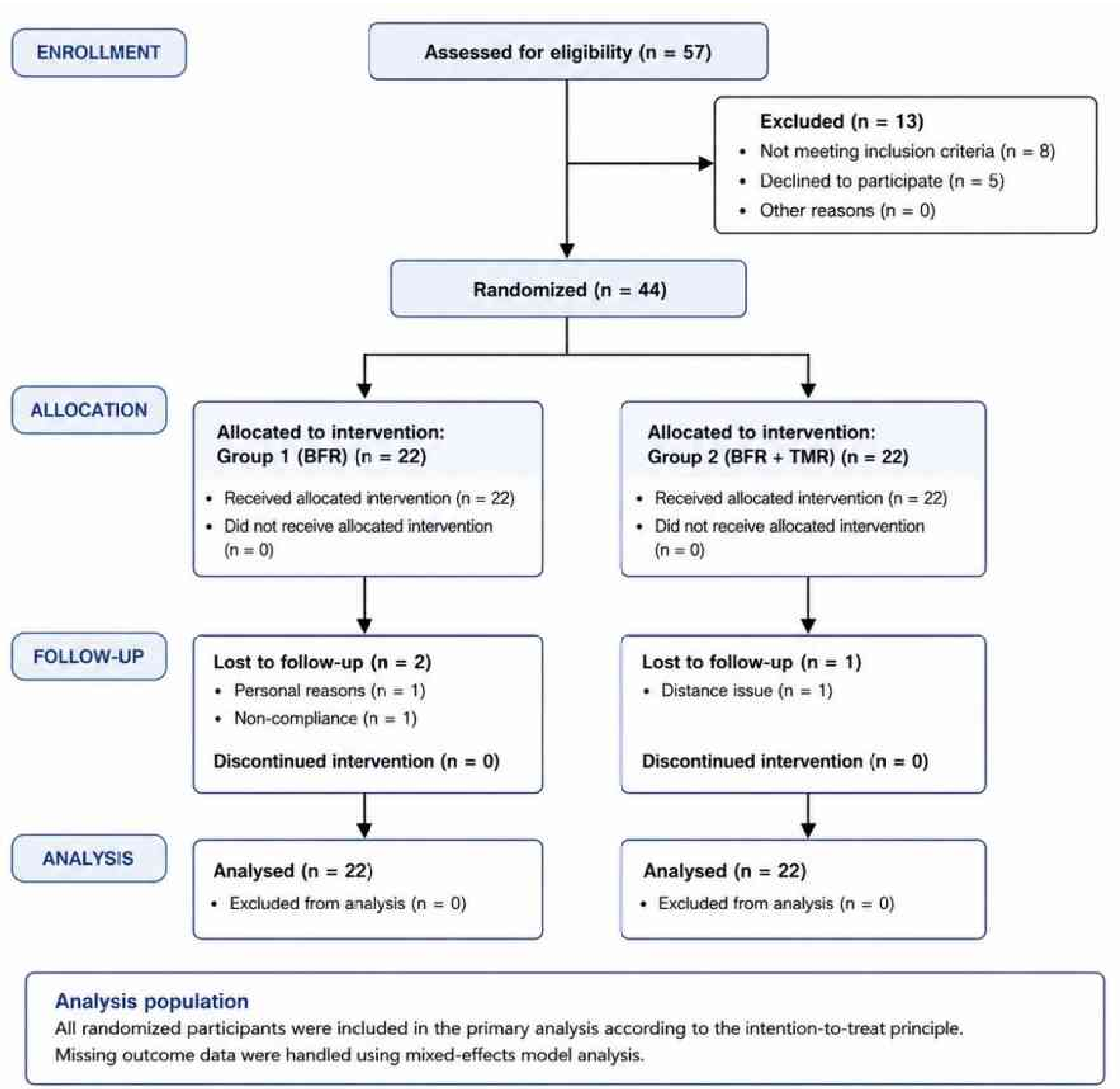

Figure 1: CONSORT flow diagram of participant recruitment, allocation, follow-up, and analysis.[17]

Table 1 shows the baseline demographic and physical characteristics. There were no statistically significant differences between groups for age, BMI, or gender distribution, indicating baseline comparability before intervention

Table 1: Comparison of Baseline Demographic and Physical Characteristics

Variable	BFR Group (n = 22)	BFR + TMR Group (n = 22)	Test Statistic	p-value
Age (years), Mean ± SD	28.45 ± 5.71	31.00 ± 5.30	t = -1.533	.133
BMI (kg/m²), Mean ± SD	23.10 ± 2.63	22.64 ± 2.36	t = 0.615	.542
Gender (Male / Female)	14 / 8	11 / 11	χ^2 = 0.834	.361
Injured Limb (Right / Left)	13 / 9	12 / 10	—	—

Note: SD = Standard Deviation; BMI = Body Mass Index; t = Independent sample t-test statistic; χ^2 = Chi-Square test statistic. For continuous variables, data are presented as Mean ± SD. For categorical parameters, data are presented as counts (frequencies).

There were no differences in age (p = .133) or BMI (p = .542) at baseline, and there was a uniform gender distribution (p = .361) as determined by independent-samples t-tests. This demographic and physical homogeneity removes potential baseline differences which would otherwise be considered confounding factors when evaluating subsequent differences in recovery rates over time, that may be confidently attributed to the different therapeutic mechanisms associated with the interventions.

3.2 Injured Limb Knee Flexion

Table 2: Descriptive Statistics and Two-Way Mixed ANOVA Results for Injured Limb Knee Flexion Range of Motion (Degrees)

Group	Time Point	N	Mean (M)	Standard Deviation (SD)	M ± SD	Two-Way Mixed ANOVA Effects
BFR	Baseline	22	63.05	5.00	63.05 ± 5.00	Main Effect for Time:
	6 Weeks	22	66.50	5.42	66.50 ± 5.42	F(3, 126) = 2088.56
	12 Weeks	22	73.86	5.48	73.86 ± 5.48	p < .001, ηp^2 = .980
	6 Months	22	93.86	5.48	93.86 ± 5.48	
BFR + TMR	Baseline	22	64.27	5.36	64.27 ± 5.36	Time × Group Interaction:
	6 Weeks	22	84.36	6.73	84.36 ± 6.73	F(3, 126) = 355.17
	12 Weeks	22	112.68	7.19	112.68 ± 7.19	p < .001, ηp^2 = .894
	6 Months	22	127.91	4.47	127.91 ± 4.47	*(Highly Significant)

Note: M = Mean; SD = Standard Deviation; ROM = Range of Motion; F = F-statistic; ηp^2 = Partial Eta Squared. Sphericity Assumed values are reported. N = 44 total participants.

Injured-limb knee flexion improved significantly over time, F(3,126) = 2088.56, p < .001, partial eta squared = .980. A highly significant Time × Group interaction was also observed, F(3,126) = 355.17, p < .001, partial eta squared = .894, confirming that the two groups followed different recovery trajectories. The BFR+TMR group increased from 64.27 ± 5.36 degrees at baseline to 127.91 ± 4.47 degrees at 6 months, whereas the BFR group increased from 63.05 ± 5.00 degrees to 93.86 ± 5.48 degrees.

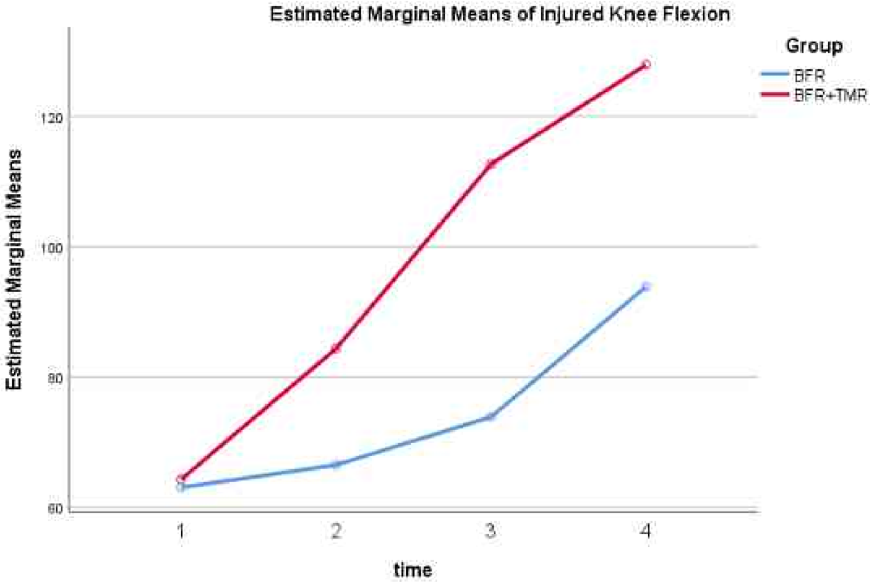

Figure 2: Mean trajectory of Injured Limb Knee Flexion Range of Motion (Degrees)

3.3 Injured Limb Knee Extension

Table 3: Descriptive Statistics and Two-Way Mixed ANOVA Results for Injured Limb Knee Extension Range of Motion (Degrees)

Group	Time Point	N	Mean (M)	Standard Deviation (SD)	M ± SD	Two-Way Mixed ANOVA Effects
BFR	Baseline	22	-25.09	2.05	-25.09 ± 2.05	Main Effect for Time:
	6 Weeks	22	-15.73	0.77	-15.73 ± 0.77	F(3, 126) = 6274.48
	12 Weeks	22	-4.68	0.84	-4.68 ± 0.84	$p < .001$, $\eta p^2 = .993$
	6 Months	22	-0.82	0.73	-0.82 ± 0.73	
BFR + TMR	Baseline	22	-24.50	1.74	-24.50 ± 1.74	Time × Group Interaction:
	6 Weeks	22	-13.73	0.77	-13.73 ± 0.77	F(3, 126) = 7.93
	12 Weeks	22	-2.68	0.72	-2.68 ± 0.72	$p < .001$, $\eta p^2 = .159$
	6 Months	22	-0.14	0.35	-0.14 ± 0.35	*(Highly Significant)

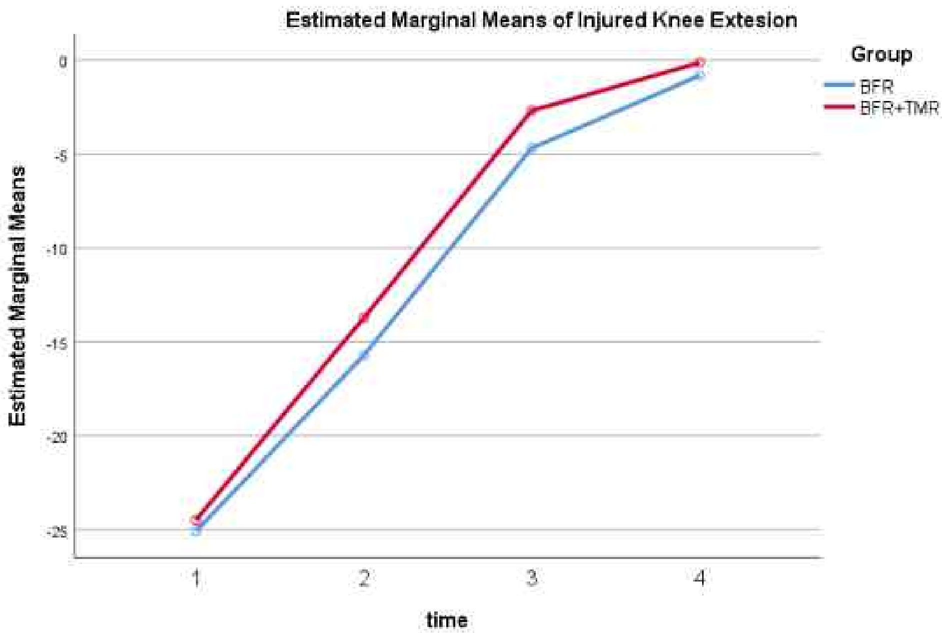

Figure 3: Mean trajectory of Injured Limb Knee Flexion Range of Motion (Degrees)

3.4 Non-Injured Knee Flexion ROM

Table 4: Descriptive Statistics and Two-Way Mixed ANOVA Results for Non-Injured Limb Knee Flexion Range of Motion (Degrees)

Group	Time Point	N	Mean (M)	Standard Deviation (SD)	M ± SD	Two-Way Mixed ANOVA Effects
BFR	Baseline	22	123.55	3.81	123.55 ± 3.81	Main Effect for Time:
	6 Weeks	22	123.68	3.62	123.68 ± 3.62	F(3, 126) = 191.89
	12 Weeks	22	123.68	3.62	123.68 ± 3.62	p < .001, ηp^2 = .820
	6 Months	22	124.00	3.27	124.00 ± 3.27	
BFR + TMR	Baseline	22	124.55	3.70	124.55 ± 3.70	Time × Group Interaction:
	6 Weeks	22	128.41	3.43	128.41 ± 3.43	F(3, 126) = 167.16
	12 Weeks	22	132.41	3.71	132.41 ± 3.71	p < .001, ηp^2 = .799
	6 Months	22	136.41	3.71	136.41 ± 3.71	*(Highly Significant)

Range of Motion (Degrees)
Non-injured limb flexion and extension also showed significant Time × Group interactions. For non-injured limb flexion, the interaction was F(3,126) = 167.16, p < .001, partial eta squared = .799. The BFR group remained almost unchanged across 6 months, whereas the BFR+TMR group increased from 124.55 ± 3.70 degrees to 136.41 ± 3.71 degrees.

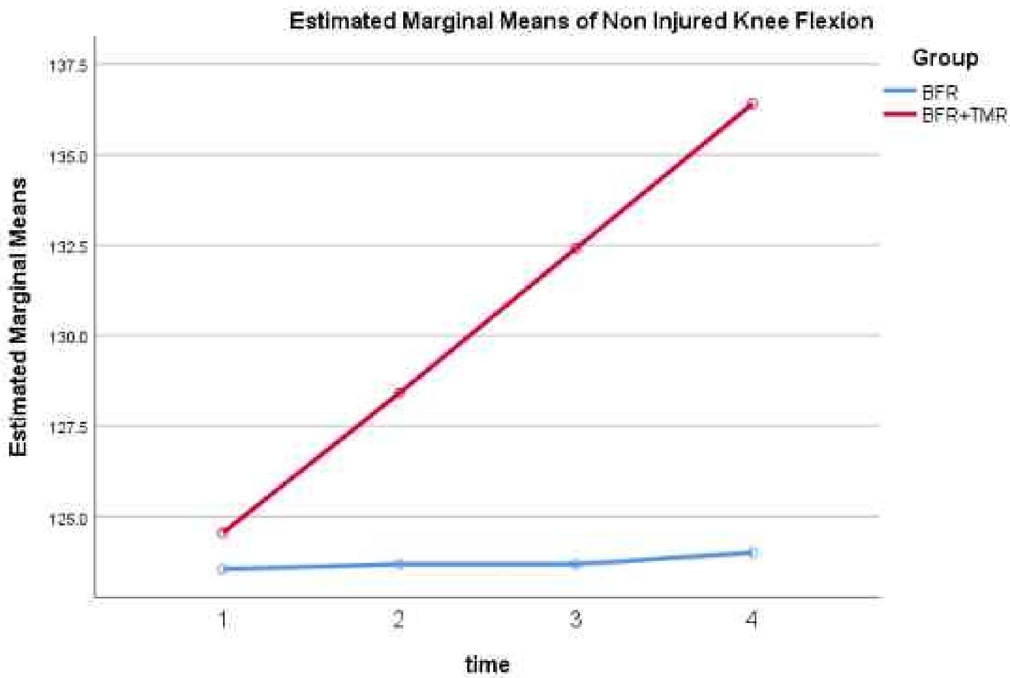

Figure 4: Mean trajectory of Non-Injured Knee Flexion

3.5 Non-Injured Knee Extension ROM

Table 5: Descriptive Statistics and Two-Way Mixed ANOVA Results for Non-Injured Limb Knee Extension Range of Motion (Degrees)

Group	Time Point	N	Mean (M)	Standard Deviation (SD)	M ± SD	Two-Way Mixed ANOVA Effects
BFR	Baseline	22	-4.91	1.38	-4.91 ± 1.38	Main Effect for Time:
	6 Weeks	22	-4.77	1.41	-4.77 ± 1.41	F(3, 126) = 121.02
	12 Weeks	22	-4.73	1.35	-4.73 ± 1.35	p < .001, ηp^2 = .742
	6 Months	22	-4.32	1.29	-4.32 ± 1.29	
BFR + TMR	Baseline	22	-4.86	1.36	-4.86 ± 1.36	Time × Group Interaction:

6 Weeks	22	-1.68	0.78	-1.68 ± 0.78	$F(3, 126) = 81.73$
12 Weeks	22	-1.00	0.82	-1.00 ± 0.82	$p < .001$, $\eta p^2 = .661$
6 Months	22	-0.14	0.35	-0.14 ± 0.35	*(Highly Significant)

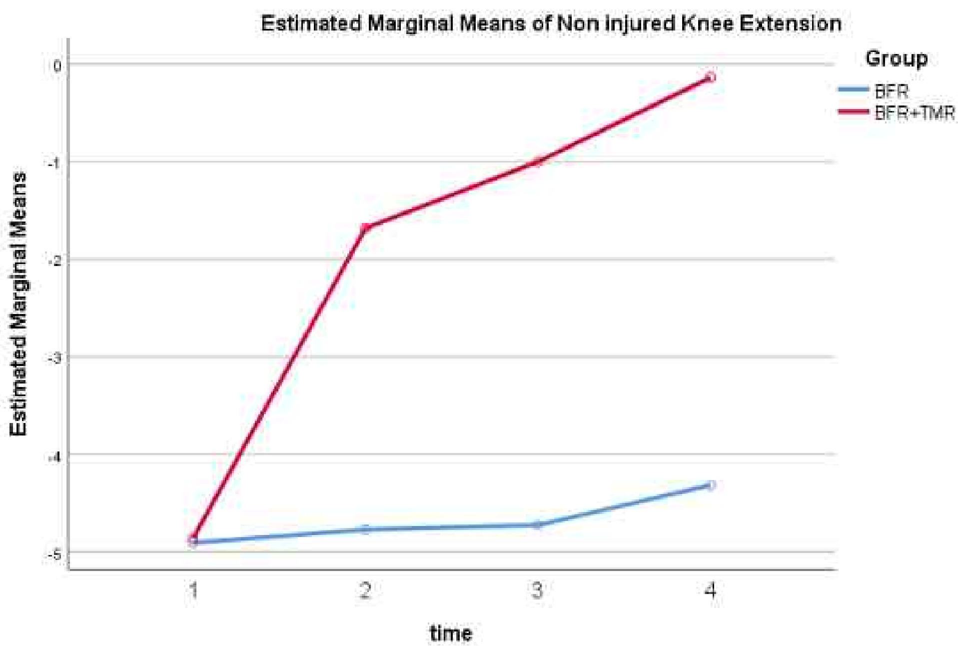

Figure 5: Mean trajectory of Non-Injured Knee Extension

4. Discussion

This was a randomized controlled trial that compared BFR with BFR + TMR following ACL injury to increase knee ROM. The key result was that both interventions led to an improvement in ROM over time, and the BFR+TMR protocol led to significantly more improvements in both injured and non-injured limb ROM outcomes. There was the greatest interaction effect for injured-limb knee flexion, suggesting that the incorporation of TMR significantly improved the recovery in the injured-limb knee flexion compared to using BFR alone. The longitudinal 6-month assessment demonstrated that while both treatments effectively supported rehabilitation, the combination of TMR and BFR led to significantly improved outcomes for subjective knee function, injured limb strength and functional balance symmetry. Crucially, baseline data demonstrated that there were no significant physical or demographic differences between groups (e.g., Age p = 0.133; BMI p = 0.542), suggesting the observed differences were a direct result of the interventions.

These results parallel current trends in rehabilitation that focus on peripheral and central mechanisms of recovery, where coordinated local strengthening and central retraining of the injured limb results in better function.[18, 19]

The injured-limb flexion findings are clinically meaningful. The BFR+TMR group reached 127.91 degrees of knee flexion at 6 months, while the BFR group reached only 93.86 degrees. This difference may influence functional performance because knee flexion is required for squatting, stair descent, kneeling, floor transfers, and sport-specific lower-limb tasks.[20] The large Time × Group interaction suggests that movement-based asymmetry correction added value beyond low-load BFR strengthening alone.

Terminal knee extension recovery is a critical marker after ACL reconstruction because residual extension deficit may affect gait mechanics, quadriceps activation, and knee joint loading. In the present study, both groups showed strong extension recovery, but the BFR+TMR group achieved closer restoration of terminal extension. Although the interaction effect for injured-limb extension was smaller than that observed for flexion, it remained statistically significant and clinically relevant because even small residual extension restrictions may influence post-operative movement quality.[10, 21]

The non-injured limb results are especially important because the BFR-only group remained nearly unchanged, while the BFR+TMR group showed clear improvement in both flexion and extension alignment. This pattern supports a possible systemic or cross-education effect of TMR. [22] Because TMR uses contralateral, pain-free, and whole-body movement strategies, it may improve global movement symmetry rather than only targeting the operated knee. These findings are consistent with the theoretical concepts of regional interdependence, central neuromuscular adaptation, and bilateral movement control.[23]

BFR remains a valuable post-operative rehabilitation tool because it can help patients exercise at low loads when high-load strengthening is not yet appropriate.[24] However, ROM recovery is not only a local muscular issue. Pain, swelling, capsular stiffness, protective movement behavior, fear of movement, arthrogenic muscle inhibition, and kinetic-chain restrictions may all contribute to limited knee motion. The current findings suggest that combining BFR with TMR may address both local tissue capacity and whole-body movement factors, resulting in superior ROM restoration.

4.1 Clinical Implications

- BFR could be considered as an option for low-load strengthening in the post-operative ACL regimen.
- The addition of TMR can help restore flexion and terminal extension recovery, which could result from protective movement patterns and movement asymmetry.
- Both injured and non-injured limbs should be monitored for ROM as contralateral changes may represent overall neuromuscular adaptation.
- The BFR+TMR method could be particularly beneficial for patients who have ongoing flexion limitation or terminal extension deficit.

4.2 Strengths of the Study

- Randomized controlled design (equal group allocation).
- Several follow-up evaluations at baseline, 6 weeks, 12 weeks and 6 months.
- ROM assessment of injured and non-injured limb.
- All ROM outcomes reported with use of inferential statistics and effect size reporting.

4.3 Limitations

- A relatively small sample size.
- Results are from one center and may not be generalizable.
- Blinding of participants and therapist was not possible, because of the nature of the interventions.
- Long-term results (after 6 months) were not assessed.
- The instrument used to measure the ROM should be clearly described and inter-rater reliability should be reported before submission of the journal.

4.4 Future Recommendations

- Larger multi-center RCTs are needed to validate these results.
- Longer follow-up periods should be evaluated in future studies to determine if ROM gains are sustained after 6 months.
- Further studies should examine the mechanisms through which TMR affects contralateral limb ROM and whole-body movement symmetry.
- Future trials should evaluate if enhanced ROM contributes to enhanced gait, return to sport readiness, strength symmetry and patient-reported outcomes.

5. Conclusion

In the post-operative ACL injury rehabilitation, the combination of Total Motion Release and BFR resulted in significantly greater improvements in knee ROM when compared with BFR alone. The BFR+TMR group exhibited greater improvements in injured-limb knee flexion recovery, more complete recovery of terminal knee extension, and greater recovery of non-injured limb range of motion. The results of this study provide clinical evidence to support the utility of implementing asymmetry correction through movement combined with low-load BFR training to maximize ROM restoration following ACL reconstruction.

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Ethical approval: Ethical approval (GIU/REC/26-30) was obtained from the Research Ethical Committee of Green International University, Lahore, Pakistan.
Informed consent: Written informed consent was obtained from all participants before enrollment.
Funding: No external funding was reported for this study.
Conflict of interest: The authors declare no conflict of interest.
Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.
Trial registration: NCT07589309

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