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Low frequency physiotherapy on joint health, hemarthrosis, walking, balance and reaction time in hemophilic arthropathy: a controlled trial

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Abstract

Background Physiotherapy is recommended for people with hemophilic arthropathy (PwHA) to improve musculoskeletal health and is typically administered in 2 or 3 sessions per week. We aimed to investigate the effects of once a week comprehensive physiotherapy and home exercise on musculoskeletal system of PwHA.

Methods In this study 19 young PwHA with knee and/or ankle were non-randomly divided into two groups: The Home Exercise Group (HEG) and the Comprehensive Physiotherapy Group (CPG). Joint health was evaluated with the Hemophilia Joint Health Score (HJHS), muscle strength (MS) with manual muscle tester, range of motion (ROM) with universal goniometer, pain with Numerical Pain Scale (NPS). The following functional tests were used: 6 min Walking Test (6MWT) for walking capacity, Functional Reach Test (FRT) for dynamic balance and Fitlight® system for visuomotor reaction time (VMRT). The frequency of hemarthrosis (FoH) in the last 6 weeks was obtained from the self-recorded data. The CPG received comprehensive physiotherapy once a week, including manual therapy, neuromuscular electrical stimulation and supervised exercises, and they performed home exercises for the other two days of the week. The HEG only performed home exercises 3 days a week. All the treatments lasted 6 weeks and evaluations were repeated.

Results Compared with the HEG, the CPG significantly ameliorated for NPS, FoH, HJHS, VMRT (time and mean), 6MWT, all MS and several ROMs. CPG significantly improved in all outcomes except for 1 ROM. The HEG significantly improved in FoH, HJHS, VMRT (time), 6MWT and MS, but not in the ROMs.

Conclusion Comprehensive physiotherapy once a week and continuing home exercise significantly improve joint health, functionality and balance, and reduce pain and hemarthrosis in PwHA.

Trial registration The study was registered at Clinicaltrials.gov (Study ID NCT06331091, retrospectively registered).

Keywords Hemophilia, Hemarthrosis, Arthropathy, Physiotherapy, Exercise, Reaction time

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Introduction

Hemophilia A is a rare congenital bleeding disorder characterized by hemarthrosis, which is mostly observed in the knee, ankle and elbow joints [1]. Recurrent hemarthrosis eventually causes hemophilic arthropathy [2]. It can reduce functionality, especially in the lower extremities [3]. Furthermore, knee and ankle joints are more prone to cartilage and bone destruction than non-weight-bearing joints [4].

In the modern hemophilia care, hemophilia-adapted physiotherapy is recommended following bleeding episodes [1]. Exercise is an important component of physiotherapy. Gradual strengthening and balance exercises are safely beneficial in people with hemophilic arthropathy (PwHA) [5, 6]. As arthropathy progresses, physiotherapy programs may be improved with appropriate manual therapy methods, electrical stimulation, and different exercise approaches [7]. Manual therapy methods increase the joint range of motion (ROM) and reduce pain in individuals with lower extremity hemophilic arthropathy [8–11]. Electrical stimulation has been shown to significantly increase muscle strength in PwHA [12]. Home exercises have positive effects on joint health and the frequency of hemarthrosis (FoH) in PwHA [10, 11]. Balance parameters improve with balance training given within the supervised physiotherapy program [13, 14]. The effect of physiotherapy on PwHA has been well investigated. Visuomotor reaction time (VMRT) is the time required to detect and respond to visual stimuli that appear in a sequential and random order [15]. Previous studies have indicated that fast VMRT of the lower extremities is associated with proper postural control, balance, and proprioception [16, 17]. Although VMRT has not been investigated in hemophilia to date, it is highly probable that VMRT is affected in PwHA and improved with physiotherapy.

Physiotherapy improves the musculoskeletal health of PwHA. PwHA spend a significant amount of time in their daily lives undergoing physiotherapy, which is typically administered 3 times a week [5, 6, 8–14]. Fewer physiotherapy sessions combined with regular home exercises may be effective. This study aimed to investigate the effects of comprehensive physiotherapy once a week in addition to home exercise compared with home exercises alone on joint health, FoH, pain, ROM, muscle strength, balance, walking capacity and VMRT.

Materials and methods

Study design and participants

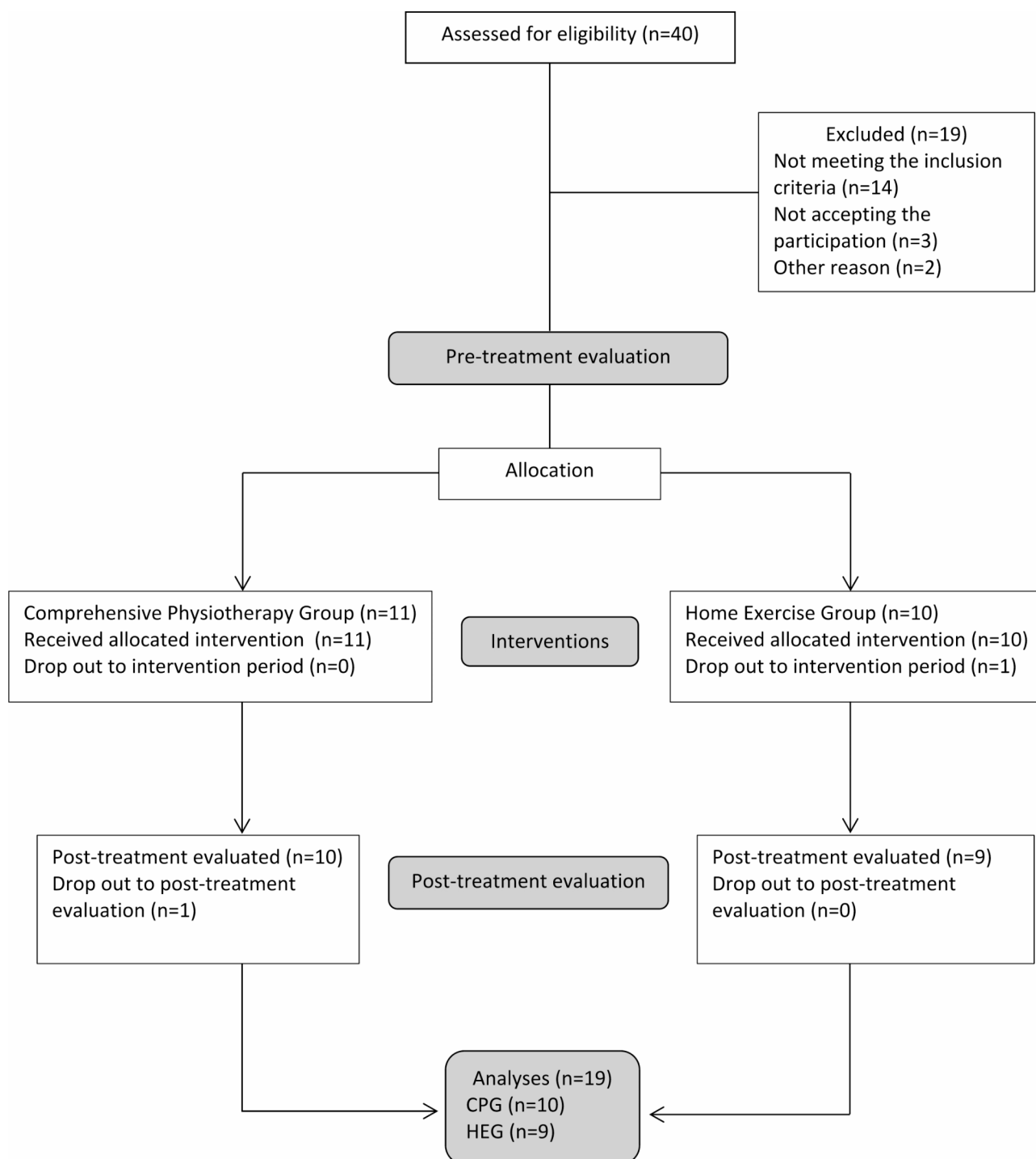
This controlled, interventional, prospective, single-center pilot study was conducted at Van Yüzüncü Yıl University, Faculty of Medicine, Department of Pediatric Hematology, between January 2023 and February 2024. This study was approved by Van Yüzüncü Yıl University, Faculty of

Medicine, Clinical Research Ethics Committee (Date: 12.10.2022, decision no:01) and was registered at clinicaltrials.gov (NCT06331091). The procedure of the study was performed according to the Declaration of Helsinki. The participants were verbally informed about the study, and written informed consent was obtained from those (over 18 years) and their parents (under 18 years). Power analysis was performed with the G-Power program (Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany) to determine the minimum number of patients to be included in the study. The joint pain parameter with an effect size of 0.77 was used for the minimum number of patients required in each group [18] and it was determined to be 8 when the type I error was $\alpha = 0.05$, the type II error was $\beta = 0.20$, and the power was 80%. The study has followed the CONSORT guidelines.

Forty PwHA with confirmed type and clinical severity of hemophilia by a hematologist were assessed for eligibility. The inclusion criteria were having severe or moderate hemophilia A, having frequent joint bleeding (at least 1 per month from the same joint in the last 6 months) and/or poor joint health (at least a knee or ankle joint score of 3 on the HJHS), receiving prophylaxis, and being adolescent or young (10–24 years). The exclusion criteria were having any neurological disease or cognitive deficit, having inhibitors, and having another orthopedic problem in the lower extremity that is not related to hemophilia, having a vision or hearing impairment that affects the proprioceptive system. Anticipating dropouts, 21 PwHA meeting the criteria were included in the study. After initial evaluation, one PwHA requested withdrawal from the study. The remaining 20 patients were divided into 2 groups: the Comprehensive Physiotherapy Group (CPG) as the intervention group and Home Exercise Group (HEG) as the control group. PwHA living in rural areas declared that it was difficult to come for treatment regularly and were requested to be assigned to HEG. Therefore, randomization was not possible, and we assigned them to HEG. One patient in the CPG dropped out because he did not attend treatments regularly. The flow chart is shown in Fig. 1.

Outcome measures

The outcome measures of the study were joint health, frequency of hemarthrosis (FoH), pain, muscle strength, ROM, balance, VMRT, and walking capacity. At the first evaluation, demographic and disease-related information (age, height, weight, hemophilia type, clinical severity, type of medical treatment, bleeding joints and FoH) was collected. For FoH, the number of hemarthrosis in the knee and ankle joints was measured in the last 6 weeks before first evaluation and at 6 weeks between the first and second evaluations during the study. The Numerical Pain Scale (NPS) was used to assess joint pain [19].

**Fig. 1** Flow diagram of study

Pain during activity (walking) and rest in the joint were assessed separately. The Hemophilia Joint Health Score (HJHS) was used to assess joint health [20]. Muscle strength was measured with a manual muscle dynamometer (Lafayette Hand-Held Dynamometer®). Knee flexors and extensors, ankle dorsi and plantar flexors were assessed [21, 22]. A universal goniometer was used to

evaluate of ROM, and the knee flexion angle, knee extension loss angle, and ankle dorsi and plantar flexion angles were measured [23]. Muscle strength and ROM measurements were made by the same physiotherapist. The Functional Reach Test (FRT) was used to assess balance [24]. HJHS, FRT, muscle strength and ROM measurements have been described in detail in previous articles

by the authors of this study [10, 11]. The VMRT of the lower extremity was evaluated with the Fitlight Trainer (FITLIGHT Sports Corp., Ontario, Canada). This wireless system consisting of 8 sensors was controlled by an application installed on a smartphone. The sensors were placed on the floor in a semicircle so that the patient could see them without turning their head while standing. The subject stood in the middle of the semicircle and the sensors were placed at a distance where the subject could comfortably reach their feet. When the measurement started, the sensor lights were turned on in a random order and the subject turned the lights off by extending his foot towards the sensor as quickly as possible without losing his balance (Fig. 2). The sensors were set to turn on a total of 20 times over 1 min. The total time, the average time it took for the light to turn off, the number of correct movements, and the number of missed movements were recorded [25, 26]. The 6-Minute Walk Test (6MWT) was used to assess walking capacity. During the test, the participants were asked to walk quickly without running in a 30-meter-long area in an empty corridor. At the end of the 6-minute period, the total distance walked by the participants was recorded in meters [27].

Intervention

After the initial evaluation, all individuals were given 60 min of education by a physiotherapist (Table 1). PwHA in the CPG and HEG performed home exercises taught by the same physiotherapist and provided in the booklet. Other treatments (manual therapy, supervised exercise and electrical stimulation) were performed by the same physiotherapist. Home exercise included warm-up, strengthening, balance and stretching exercises [10, 11]. The exercise protocol was given Appendix 1. All individuals were given a resistance band appropriate for their muscle strength and joint health (yellow, red or green resistive exercise band). The PwHA in the CPG received comprehensive physiotherapy in the hospital one day a week, which included electrical stimulation, manual therapy, and supervised exercise (Appendix 1). Electrical stimulation was applied to the knee extensors and ankle dorsiflexors (one or both extremities with arthropathy) [12]. High voltage pulsed galvanic stimulation is preferred because it provides high patient comfort [28]. In manual therapy, myofascial release techniques for soft tissue, and traction and grade I-II joint mobilization for restricted knee and/or ankle joints were applied [9, 11]. The CPG performed the same exercises given to the HEG under supervision in the clinic one day a week and at home the other 2 days a week. The regular use of prophylaxis and home exercise was confirmed for HEG who were followed by phone calls. For this study, patients' prophylaxis days were not changed. PwHA received

prophylaxis on Mondays/Thursdays or Tuesday/Friday. Two days of intervention were arranged on these days. The third intervention day was not a prophylaxis day but was immediately following the second day. All the treatments lasted 6 weeks, and the evaluations were repeated at the end of this period.

Statistical analysis

The data of the study were analyzed with IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp. Armonk, NY, USA) and were studied at 95% confidence interval ($p=0.05$). Shapiro-Wilk's test ($n<50$) was used for normality testing. Descriptive statistics of variables showing normal distribution were presented as mean and standard deviation; those of data without a normal distribution were given as median, minimum-maximum. The frequency and percentage values of non-numerical parameters were given. Paired group comparisons showing normal distribution were compared with Independent samples t test, and variables not showing normal distribution were made with Mann-Whitney U test.

Results

This study included 21 PwHA and was completed with second evaluations of 19 PwHA. The mean age, height, weight and BMI of PwHA and groups were given Table 1. Among the 19 PwHA, 15 (79%) had severe hemophilia and 4 (21%) had moderate hemophilia. PwHA had HA symptoms in a total of 15 knee joints, 20 ankle joints, and 10 elbow joints. The numbers of knee and ankle joints with HA were 18 and 17 in CPG and HEG, respectively (Table 2). There was no statistically significant difference between the groups in terms of age, number of joints with arthropathy or the clinical severity of hemophilia ($p<0.05$) but not in height, weight or BMI ($p>0.05$). PwHA did not experience hemarthrosis during or the day following exercise.

Outcome measurements

The results of the outcome measurement were presented Table 3. Rest pain of all PwHA was found to be 0. Activity pain data were only given Table 2 as NPS. Intra-group and inter-group comparisons of the pre- and post-treatment NPS, FoH, FRT, HJHS, VMRT (time and mean) and 6MWT data of the CPG and HEG are given in Table 2. There was a significant improvement in all measurements in intra group comparison of CPG ($p<0.05$). In the HEG, there was a significant amelioration in FoH, HJHS, VMRT (time) and the 6MWT ($p=0.024$, 0.042 , 0.044 , 0.036 , respectively) but not in the NPS. In the intergroup comparison, the CPG was found to be significantly superior to the HEG in all measurements ($p<0.05$) except for the FRT (Table 2).



Fig. 2 Test of visuomotor reaction time

Table 1 Clinical and demographic characteristics of study groups and the all patient with haemophilia in the study

		All PwHA (n = 19)	CPG (n = 10)	HEG (n = 9)	p
Age (y)		18.3 ± 7.2	20.2 ± 7.4	16.2 ± 6.6	0.241
Height (cm)		160 ± 15.3	166.6 ± 12.6	152.6 ± 15.2	0.044
Weight (kg)		53.9 ± 16.6	62.2 ± 14.1	44.7 ± 14.6	0.016
BMI (kg/cm ²)		20.6 ± 3.6	22.2 ± 3.4	18.7 ± 2.9	0.028
Joint with arthropathy	Knee	15 (%33.3)	7 (30%)	8 (36%)	0.125
	Ankle	20 (%44.4)	11 (48%)	9 (41%)	0.237
	Elbow	10 (%22.2)	5 (22%)	5 (23%)	0.95
Hemophilia severity	Moder- ate	4(21%)	2(20%)	2(22%)	0.89
	Severe	15(79%)	8(80%)	7(78%)	0.85

Abbreviations: PwHA, Patient with hemophilic arthropathy; CPG, Comprehensive Physiotherapy Group; HEG, Home Exercise Group; BMI, Body Mass Index. Independent Sample t Test, mean ± standard deviation and number (percentage), $p < 0.05$: significant

ROMs of the knee and ankle joints with arthropathy in the CPG and HEG were compared (Table 3). In the CPG, there was a significant difference in all ROMs ($p < 0.05$) except for loss of extension of knee ($p = 0.065$) between pre- and post-treatment but not in HEG ($p > 0.05$). In

addition, the CPG was superior to the HEG terms of the flexion angle of knee ($p = 0.04$) and plantar flexion angle of the ankle ($p = 0.005$).

The muscle strength of flexor and extensor of knee and dorsi and plantar flexion was given in Table 4. There was a significant increase in all muscle strength in post-treatment compared to pre-treatment both the CPG ($p \leq 0.001$) and the HEG ($p < 0.05$). However, the increase in CPG was significantly greater than that in HEG ($p < 0.05$).

Discussion

It was shown that comprehensive physiotherapy once a week with home exercise was significantly superior to home exercises alone in terms of ameliorating the joint health, number of hemarthrosis, activity pain, muscle strength, ROM, walking capacity, and visuomotor reaction time in PwHA with frequent hemarthrosis or poor joint health.

Studies have shown that different physiotherapy methods have positive effects on the joint health of PwHA. In these studies, physiotherapy was generally applied

Table 2 Intra-group and inter-group comparisons of pre- and post-treatment values of some outcome measures

	CPG (n = 10)			HEG (n = 9)			CPG	HEG	p
	t0	t1	p	t0	t1	p	t0-t1	t0-t1	
NPS (0–10)	6.5 (0–7)	3 (0–4)	0.016	0(0–7)	0(0–6)	0.317	-3(-4-0)	0(-7-0)	0.035
FoH (number)	3.5 (0–6)	0 (0–2)	0.007	2(0–3)	0(0–2)	0.024	-3(-5-0)	-1(-2-0)	0.044
HJHS (0-124)	15.5 (4–52)	12.5(1–49)	0.005	2(0–40)	2(0–37)	0.042	-3(-8–2)	-1(-6-0)	0.009
FRT (cm)	33.2 (26–51)	38.25 (32–52)	0.005	29 (21–48)	36(23.5–41.5)	0.109	4.3 ± 3	2.4 ± 4.1	0.260
VMRT (s)	24.7 (22.9–32.2)	22.93 (19.8–25.3)	0.008	25.45 (23.5–29.1)	24 (22.05–28.9)	0.044	-3.4 ± 2.5	-1.1 ± 1.3	0.024
Time Mean	0.8 (0.65–1.2)	0.68 (0.5–0.8)	0.008	0.8 (0.7-1)	0.8 (0.65-1)	0.058	-0.2 ± 0.1	0 ± 0.1	0.024
6MWT (m)	546 (432–645)	603 (480–704)	0.005	518 (358–660)	537 (358–670)	0.036	56 (11–101)	10 (-6-37)	0.003

Abbreviations: CPG, Comprehensive Physiotherapy Group; HEG, Home Exercise Group; NPS, Numeric Pain Scale; FoH, Frequency of Hemarthrosis; HJHS, Hemophilia Joint Health Score; FRT, Functional Reach Test; VMRT, Visuomotor Reaction Time; 6MWT, 6 min Walking Test; t0, pre-treatment; t1, post-treatment. Median (Min-Max), p : Wilcoxon Test (Intragroup comparison). Mean ± Std.Dev, p : Independent Sample t Test, Median (Min-Max), p : Mann-Whitney U Test (Intergroup comparison). $p < 0.05$ significant

Table 3 Intra- and inter-group comparison of pre- and post-treatment values of range of motion of knee and ankle joints with arthropathy in groups

	CPG (n = 18)			HEG (n = 17)			CPG	HEG	p
	t0	t1	p	t0	t1	p	t0-t1	t0-t1	
KNEE	n = 7			n = 8			n = 7	n = 8	
LEA (°)	0 (0–18)	0 (0–12)	0.065	0(0–34)	0(0–32)	0.1	0(-8-0)	0(-6-0)	0.22
FA (°)	123(88–132)	128(90–137)	0.015	130(94–140)	130(94–140)	0.1	5(0–13)	0(0–12)	0.04
ANKLE	n = 11			n = 9			n = 11	n = 9	
DFA (°)	10(0–20)	13(6–25)	0.021	18(-4-25)	20(2–25)	0.08	4(-2-10)	0(0–6)	0.08
PFA(°)	31.5(22–50)	38.5(28–52)	0.007	45(30–50)	45(35–50)	0.08	5(-2-13)	0(0–6)	0.005

Abbreviations: LEA, Loss of Extension Angle; FA, Flexion Angle; DFA, Dorsi Flexion Angle; PF, Plantar Flexion Ankle. t0, pre-treatment; t1, post-treatment. Mean ± Std.Dev, p : Independent Sample t Test, Median (Min-Max), p : Mann-Whitney U Test (Intergroup comparison). $p < 0.05$ significant

Table 4 Intra-group and inter-group comparisons of pre- and post-treatment values of right and left extremity muscle strength

	CPG (n = 20)			HEG (n = 18)			CPG	HEG	P
	t0	t1	p	t0	t1	p	t0-t1	t0-t1	
KE MS (lbs)	12 (4–35)	14 (7–37)	0,001	8 (4–19)	10 (6–18)	0,002	3 (-4–11)	1 (-1–4)	0,047
KF MS (lbs)	14 (7–27)	19 (10–32)	< 0,001	11 (6–25)	13 (7–30)	0,014	4 (0–10)	1 (-6–5)	< 0,001
ADF MS (lbs)	13 (8–35)	18 (10–40)	< 0,001	12 (7–29)	13 (10–29)	0,044	3 (0–11)	1 (-4–5)	0,011
APF MS (lbs)	13 (7–32)	21 (13–34)	< 0,001	10 (7–28)	11 (9–28)	0,001	6 (1–11)	2 (0–5)	< 0,001

Abbreviations: CPG, Comprehensive Physiotherapy Group; HEG, Home Exercise Group; KE, Knee Extensor; KF, Knee Flexor; ADF, Ankle Dorsi Flexor; APF, Ankle Plantar Flexor; MS, Muscle Strength. t0, pre-treatment; t1, post-treatment. Median (Min-Max), p: Wilcoxon Test (Intragroup comparison); p: Mann-Whitney U Test (Intergroup comparison); p < 0.05 significant

3 times a week. This study addressed the frequency of physiotherapy for the first time and showed that less frequent physiotherapy may be effective. In this study, manual therapy, electrical stimulation and exercise (supervised and home) were given together in the intervention group (CPG) as comprehensive physiotherapy. Although these methods are generally examined separately in scientific research, they are often used together in physiotherapy clinics. Therefore, this study provides a clinically significant result. The control group (HEG) was given only home exercises. As a result, comprehensive physiotherapy once a week and home exercises were significantly superior to only home exercises in almost all outcome measurements.

In PwHA, hemarthrosis is more common in the lower extremity than in the upper extremity [30]. It has been reported that the ankle bleeds more than the knee and elbow [31]. In this study, the joints with the most frequent bleeding were examined, and the ankle was the first affected joint, with a rate of 44%. The rates for the knee and elbow joints were 33% and 22%, respectively. These results show that the lower extremity is more prone to bleeding, which is consistent with the literature and supports the idea that this study is lower extremity-based. In addition, a significant difference was observed between the groups in terms of height, weight and BMI. However, the differences between the pre- and post-treatment were compared between the groups. It was thought that this statistical difference did not have a significant effect on the study results.

The FoH and HJHS, were significantly improved after treatment in both CPG and HEG compared to pre-treatment. However, CPG was found to be significantly superior to HEG. In addition, the reason why FoH decreased significantly in all patients may be that PwHA were under the supervision and responsibility of a physiotherapist during the study. Thus, PwHA may have been more careful about prophylaxis, taking precautions against trauma, and exercising.

The FRT, a balance parameter, improved significantly only in the CPG after treatment compared with before treatment. We report that manual therapy and supervised exercise improve dynamic balance. In a study conducted on individuals with ankle limitations, manual therapy was reported to improve balance parameters [32]. A meta-analysis study reported that joint mobilization as a manual therapy technique improved dynamic balance in the short term after application, but further studies are needed in the long term [33].

The VMRT measures the duration of the motor response to a visual stimulus and requires proper postural control, balance, and proprioception. VMRT increased significantly in the CPG (time and mean) and HEG (only time), and the increase in the CPG was significantly greater than that in the HEG. Manual therapy may improve joint kinetics and kinematics, potentially influencing proprioception. In a study, it was stated that manual therapy increased proprioception in the short term, and various types of exercises were needed to increase proprioception in the long term [34]. In this current study, both exercise and manual therapy were applied to the CPG and VMRT was developed.

The activity pain decreased significantly only in the CPG. It can be said that techniques such as joint mobilization and soft tissue mobilization applied in manual therapy are effective in reducing pain. A meta-analysis of knee osteoarthritis, it was reported that manual therapy is effective in reducing pain and increasing functionality, and that it may be more effective in applications lasting 4 weeks or more [35]. In this study, it was thought that a 6-week manual therapy contributed to a significant reduction in activity pain.

The walking capacity assessed with the 6MWT increased significantly in both the HEG and the CPG. However, the increase in CPG was significantly greater than the increase in HEG. The increase in walking distance is probably related to the reduction in activity pain. In addition, a study reported that a supervised exercise

program improved walking capacity and joint health in PwHA (37).

According to the ROM results, knee flexion, ankle dorsi and plantar flexion angles increased significantly in the CPG, whereas there was no significant increase in any of the angles in the HEG. One of the most important effects of manual therapy is to increase the restricted ROM [8–11]. The reason for the ROM increase in the CPG is manual therapy. Since there was no significant increase in the ROM of individuals in HEG, it can be said that home exercises are insufficient to increase ROM.

In the evaluation of muscle strength, the exercise program provided a significant increase in all muscle strengths of the both the CPG and HEG. However, the increase in CPG was significantly greater than that in HEG. This difference is thought to be due to the supervised exercise and electrical stimulation applied to the CPG once-a-week. There are very few studies in the literature that have applied electrical stimulation to PwHA to increase muscle strength and have shown that electrical stimulation (three times) a week is safe and effective (12,38). In our study, we thought that electrical stimulation addition exercise may be beneficial even if it is applied once a week.

The most important limitation of this study is the inability to compare the CPG with the routine 3-session-per-week physiotherapy group due to case limitations. Second, randomization and blinding could not be applied. Randomized controlled blinded trials evaluating specific physiotherapy protocols and methods are recommended for future studies.

Conclusion

Once-weekly comprehensive physiotherapy with home exercises significantly improved joint health, hemarthrosis, pain, strength, ROM, walking capacity, balance, and VMRT in PwHA compared to home exercises alone. Additionally, only home exercises, prescribed and followed by a physiotherapist, significantly improved in most of the evaluated outcome measures, including hemarthrosis. It is thought that receiving physiotherapy even if infrequently and continuing home exercises may be an effective approach to improve the musculoskeletal health of PwHA. In addition, this protocol may be especially useful for PwHA who have difficulty adapting to frequent physiotherapy sessions. However, randomized controlled trials with larger sample sizes are needed.

Abbreviations

PwHA	Patient with hemophilic arthropathy
FoH	Frequency of hemarthrosis
ROM	Range of motion
VMRT	Visuomotor reaction time
FRT	Functional Reach Test
CPG	Comprehensive Physiotherapy Group
HEG	Home Exercise Group

BMI Body mass index

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12891-025-08549-4>.

Supplementary Material 1

Supplementary Material 2

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Author contributions

AMT contributed to the experimental study and writing manuscript. NMT contributed to the design of the study and data collection. SS and MB contributed to application of treatments and data collection. KK contributed to the experimental study. All authors reviewed the manuscript.

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Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by Van Yüzüncü Yıl University, Faculty of Medicine, Clinical Research Ethics Committee (Date: 12.10.2022, decision no:01). The procedure of the study was performed according to the Declaration of Helsinki. The participants were verbally informed about the study, and written consent was obtained from those who volunteered.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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