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Comparative effects of dual-task training versus combined exercise training in water and on land on patients with multiple sclerosis: A Study protocol of a randomized factorial trial

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Note from the Editors: Instructions for reviewers of study protocols

Since launching in 2011, BMJ Open has published study protocols for planned or ongoing research studies. If data collection is complete, we will not consider the manuscript.

Publishing study protocols enables researchers and funding bodies to stay up to date in their fields by providing exposure to research activity that may not otherwise be widely publicised. This can help prevent unnecessary duplication of work and will hopefully enable collaboration. Publishing protocols in full also makes available more information than is currently required by trial registries and increases transparency, making it easier for others (editors, reviewers and readers) to see and understand any deviations from the protocol that occur during the conduct of the study.

The scientific integrity and the credibility of the study data depend substantially on the study design and methodology, which is why the study protocol requires a thorough peer-review.

BMJ Open will consider for publication protocols for any study design, including observational studies and systematic reviews.

Some things to keep in mind when reviewing the study protocol:

- Protocol papers should report planned or ongoing studies. The dates of the study should be included in the manuscript.
- Unfortunately we are unable to customize the reviewer report form for study protocols. As such, some of the items (i.e., those pertaining to results) on the form should be scores as Not Applicable (N/A).
- While some baseline data can be presented, there should be no results or conclusions present in the study protocol.
- For studies that are ongoing, it is generally the case that very few changes can be made to the methodology. As such, requests for revisions are generally clarifications for the rationale or details relating to the methods. If there is a major flaw in the study that would prevent a sound interpretation of the data, we would expect the study protocol to be rejected.

Comparative effects of dual-task training versus combined exercise training in water and on land on patients with multiple sclerosis: A Study protocol of a randomized factorial trial

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Abstract

Introduction: People with multiple sclerosis (PwMS) experience cognitive and motor impairments, including decreased processing speed, reduced attentional demands, impaired balance, and gait dysfunction. Previous research underscores the potential of exercise training as a non-pharmacological strategy to ameliorate these impairments in PwMS. We are conducting a study to compare the effects of dual-task training and combined exercise training in water and on land on the cognition, balance, and gait of PwMS.

Methods and analysis: This is a double-blind 3*2*2 factorial randomized trial. Participants will be randomly allocated to one of six groups: four intervention groups and two control groups. All patients will receive supervised 12-week training sessions, twice a week, and will be assessed by a blinded outcome assessor before and at the end of the 12-week program. The primary outcome includes The Symbol Digit Modalities Test (SDMT). The secondary outcomes involve the California Verbal Learning Test-II (CVLT-II), the Brief Visuospatial Memory Test-Revised (BVMT-R), kinetics, and kinematics throughout the balance and gait cycle. All the data will be analyzed by a blinded data analyst.

Ethics and dissemination: Ethical approval was granted by the Sports Science Research Institute (SSRI) (No. IR.SSRC.REC.1401.082). The results of the trial will be submitted for publication in journals and distributed to PwMS and physiotherapists.

Trial registration number: The trial is prospectively registered on 2024/03/22, at https://www.umin.ac.jp/ with an identification number (UMIN000053947).

Keywords: Dual-task training, Combined exercise training; Multiple sclerosis; Cognition; Gait; Balance.

Strengths and limitations of this study

► This study protocol describes the combined and dual-task exercise training in two different environments for People with Multiple Sclerosis (PwMS) to assess cognitive and motor movements.

► The study assesses cognitive and motor movements, targeting the enhancement of daily activities in PwMS.

► Increasing awareness can enhance therapeutic interventions and symptom management for PwMS, potentially aiding healthcare professionals in developing more effective exercise interventions for PwMS.

► Physical activity and cognitive impairments will be assessed using the cognitive assessment tool and kinetics and kinematics measurements throughout the balance and gait cycle.

► Participants and physical therapists will not be blinded to group allocation.

Introduction

Multiple sclerosis (MS) is a neurologic disease that adversely affects the central nervous system (CNS) through inflammation and demyelination. More than 2.8 million people worldwide are affected by MS, with a higher prevalence among women than men (1). One of the most common phenotypes of MS is relapsing-remitting (RRMS) (2). RRMS is typically diagnosed when PwMS have lesions in their nervous system or experience intermittent episodes of neurological symptoms involving inflammation over time (3), leading to demyelination, axon injury, loss of nerve cells, and subsequent atrophy (4). Hence, PwMS often experiences a range of symptoms, including cognitive issues and motor dysfunctions. Studies investigated that 40- 65% of PwMS and 30-45%

of individuals with RRMS exhibit cognitive impairment (5, 6). Common symptoms of cognitive impairment include decreased processing speed, deficits in learning and memory, perceptual skills, executive functions, and rarely linguistic or intellectual impairment (7). Cognitive domains are crucial for executing motor tasks smoothly and managing complex actions. For instance, processing speed is vital for efficient signal transmission between the CNS and motor movement. Additionally, it predicts performance in executive functions and daily activities (8). Executive functioning encompasses cognitive skills required for engaging in complex, goal-directed behavior and adapting to environmental demands, including abilities like planning and anticipating outcomes(8). Therefore, deficits in cognitive domains can significantly impair motor function and the performance of daily activities in PwMS.

Due to the interaction between decreased physical and cognitive abilities, up to 75% of PwMS experience issues with balance and gait, both in the early stages and advanced stages of the disease (9). Balance problems can also result from CNS damage or abnormal gait, leading to falls, injuries, loss of mobility, and reduced quality of life (10). Moreover, there is evidence that balance depends not only on the integration of somatosensory, visual, and vestibular information but also on higher brain systems responsible for the memory needed for anticipatory movement(11).

Several studies have highlighted the walking difficulties in PwMS; Benedetti et al. reported that PwMS had a slower progression pace, shorter steps, and prolonged double support intervals during walking (12). Coca-Tapia et al. showed a decrease in speed, step lengths, and stride lengths, alongside increased step width. They also noted decreases in hip extension during the stance phase, reduced knee flexion during the swing phase, decreased ankle dorsiflexion at initial contact, and decreased ankle plantar flexion during the preswing phase (13).

The decline in cognitive and physical performance significantly affects the daily activities and independence of PwMS (14), underscoring the critical role of symptom management. Managing symptoms is fundamental for promoting health and well-being in PwMS (15). Exercise training is a valuable complement to disease-modifying drugs for managing MS and its symptoms (16), highlighting the need for effective non-pharmacological treatments like exercise therapy.

Unlike single-motor tasks, which may not fully represent daily activities, dual-tasks better simulate the cognitive and motor challenges of daily life (17). Therefore, evaluating dual-task performance and tracking improvements can provide valuable insights into the overall functional status of PwMS. For instance, Kessler et al. found that difficulties in new learning significantly impacted activities of daily living, including shopping, housework, and transportation (18). Additionally, safe functional movements necessitate simultaneous motor and cognitive tasks which are referred to as dual-task (19). PwMS exhibit a reduced ability to perform dual-tasks (20), even in those with low EDSS scores (21). It has been demonstrated that DTT has a moderately positive impact on enhancing dynamic balance and functional mobility in PwMS (22). Additionally, it can improve gait speed, motor performance, and cognitive function in PwMS (17, 23).

Previous research indicates that aquatic training confers notable advantages over land-based training in MS research, notably due to buoyancy, viscosity, and thermodynamics (24). PwMS commonly experience pain and reduced mobility (24). Buoyancy mitigates joint impact, facilitating movements that may be challenging on land (25). Also, Buoyancy mitigates muscle weakness and reduces the risk of fall-related injuries and fractures, prevalent among PwMS (26). Studies have demonstrated that participating in aquatic activities can enhance muscle strength and reduce fatigue, both common concerns for PwMS (24, 27). Viscosity provides drag forces, allowing multi-directional performance and offering low-impact resistance training, which can improve muscle strength and endurance (24, 27). Land-based training also improves resistance; however, it can be adjusted by using weights or resistance bands.

Cerebral blood flow issues in PwMS lead to cognitive impairment, lesion formation, axonal degeneration, and fatigue (28). Immersing in water may boost blood flow through hydrostatic pressure, stimulating mechanoreceptors, and increasing parasympathetic activity through a baroreflex triggered by the rise in central blood volume and stroke volume. Thus, Being partially submerged may also enhance cognitive function (29, 30). Thermodynamics is advantageous due to the Uhthoff phenomenon, wherein MS symptoms exacerbate with increasing temperatures (31). Water conducts heat 25 times faster than air, leading to more efficient regulation of body temperature during aquatic training compared to land-based exercises, due to water's superior heat retention capacity and faster heat transfer properties (24). Previous studies have demonstrated the benefits of various cooling strategies before and after exercise training for PwMS (24, 32). Among these strategies, engaging in aquatic exercise training in a cool pool stands out as an effective method to mitigate heat sensitivity.

Multiple studies have investigated the effectiveness of various exercise training interventions on in PwMS (26, 27, 33, 34). However, a notable limitation is the tendency to focus on a singular mode of exercise training, neglecting the multifaceted physiological decline (e.g., aerobic deconditioning, muscle weakness, and balance dysfunction) in PwMS (35). In addition to the previous emphasis on addressing balance deficiencies in PwMS (10), there is evidence suggesting that PwMS also experience decreases in both muscular and cardiorespiratory fitness levels. Lambert et al. found that PwMS exhibited significantly lower peak torque in various leg muscles compared to healthy individuals (33). Similarly, Moatert et al. observed that cardiorespiratory fitness, measured by VO2peak, was 28% higher in healthy controls than in PwMS (34). The decreasing fitness levels in PwMS, coupled with the vital importance of physical fitness for improving functionality and managing symptoms, highlight the critical need for concurrent interventions improving balance, gait, muscle strength, cardiovascular health, and cognitive functions (36-38). Accordingly, Sandroff et al. (36) demonstrated that engaging in multimodal exercise training could enhance endurance walking performance and cognitive processing speed in PwMS experiencing significant mobility limitations. This improvement is likely attributed to enhancements in cardiorespiratory capacity. Motl et al. linked aerobic fitness with increased volumes of subcortical grey matter structures such as the hippocampus and basal ganglia(39), possibly explaining exercise's positive impact on ambulation and cognition(40). We will therefore conduct a 12-week factorial trial to evaluate the effect of DTT and combined exercise training in water and land, on cognition, balance, and gait in PwMS.

Aims and hypothesis

The primary objective of this study is to compare the effects of DTT and combined exercise training in water and on land on cognitive impairment in PwMS and to examine the interaction effects of these treatments. Evaluating the effects of these interventions on gait kinetics and kinematics, including spatiotemporal parameters, sagittal plane angles, and moments of the hip, knee, and ankle, as well as anteroposterior, mediolateral, and vertical ground reaction forces in two different environmental conditions, remains secondary. The hypothesis posits that aquatic environments may enhance cognition and motor function more effectively than land environments due to increased cerebral blood flow, mechanoreceptor stimulation, and potential parasympathetic activity. This is supported by evidence indicating that water immersion could mitigate cognitive impairment and improve neural pathways and motor function. Another hypothesis suggests that combined exercise training may have a greater effect on gait kinetics compared to DTT. This is based on the importance of balance enhancement for improving gait, proprioception, coordination, and postural stability. Strengthening muscles is crucial for maintaining alignment and restoring the ability to respond quickly to stimuli, which can positively affect gait dynamics. Furthermore, aerobic exercises can enhance cardiovascular health, potentially increasing endurance during walking.

Method and Analysis

Study Design

This is a 2*2*3 factor randomized study with double-blind outcome assessor and the data analyst conducted at the campus of Kharazmi University, Tehran, Iran. The protocol is described according to Recommendations for Interventional Trials (SPIRIT) guidelines (Table 1), and the results of the study will be presented in a CONSORT statement (Fig. 1).

Table 1. SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) diagram of enrolment, interventions, and assessments of the trial.

	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
TIMEPOINT	**$-t_1$**	**0**	**Week 4**	**Week 8**	**Week 12**	**t_x**
Enrolment						
Eligibility screen	X					
Informed consent	X					
Cognitive impairments assessment	X					
Safety for exercise clearance	X					
Allocation		X				
Interventions						
Dual-task in water						
Dual-task on land						
Combined in water						
Combined on land						

Control in water						
Control on land						
Clinical measures						
Weight, Height, BMI		X				
Level of education		X				
Symptoms duration		X				
Diagnosis duration		X				
Medication use		X				
Assessment						
BICAMS		X				X
Gait characteristics		X				X
Balance assessment		X				X
Safety (AEs)	X	X	X	X	X	X
Early withdrawal information	As required					
AEs: adverse events, BICAMS: Brief International Cognitive Assessment for MS						

Patient and public involvement

The study is crafted through extensive cooperation among PwMS, healthcare experts, and researchers. Additionally, representatives from the Alborz MS NGO of Iran, who are also PwMS, are involved as project partners to guarantee the benefits for patients.

Eligibility criteria

Participants are eligible to be included in the trial if they meet the following inclusion criteria: PwMS will be aged between 20 and 60, including both males and females, diagnosed with relapsing-remitting MS as outlined by the McDonald criteria (41), the EDSS scores ranging between 1.0 and 5.5 (42), Relapse-free for the last 3 months (43), have not engaged in regular physical exercise (no training within the last three months), be willing to participate in the current study, Cognitive impairments that will be characterized by scores below the standard criterion of at least 1.5 standard deviations (8) on at least one assessments from the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) (44).

Potential participants will be excluded if they are unable to comply with the requirements of the protocol, inability to stand and walk without aids and braces, changes in medication in the past 2 months (45), history of cardiovascular, kidney, or other chronic diseases, pregnancy in the past 12 months (45), medical conditions interfering with mobility, MS-like syndromes such as neuromyelitis optica, or major problems with hearing, vision, and perception(46).

Recruitment

We will recruit PwMS from the Alborz MS NGO of Iran. Community-based recruitment will be accomplished through collaboration with PwMS, neurologists, advertising, social media platforms (e.g., Instagram), and hospital boards within the area (Alborz, Karaj). Based on the inclusion criteria, the researchers will enroll eligible PwMS in the study. At enrollment, contributors will be randomly assigned to one of six groups: dual-task training in water, dual-task training on land, combined exercise training in water, combined exercise training on land, control group in water, and control group on land (Fig. 2).

Screening

Volunteers will undergo a telephone assessment to determine eligibility. Those meeting the criteria will be invited for a screening appointment at the trial center. This includes MS EDSS evaluation, medication history questionnaires, exercise safety clearance, assessment of baseline physical activity, and evaluation for cognitive impairment by a neurologist.

Safety for Exercise Clearance

A pre-exercise assessment will identify PwMS at higher risk of adverse events during exercise. Functional assessments will be utilized to develop exercise program recommendations, aligned with American Sports Medicine Association guidelines (47). These include the six-minute walk test (6MWT), where patients walk as fast as they comfortably can for 6 minutes without running or jogging, aimed at measuring functional endurance (48). The timed 5-repetition sit-to-stand assesses the time taken to complete five repetitions of the sit-to-stand movement as quickly as possible, serving as a measure of strength (49). Furthermore, the Berg Balance Scale evaluates patients' performance on 14 items, comprising 5 static and 9 dynamic items, related to balance function frequently encountered in everyday life (50).

Safety

We will monitor adverse events (AEs) during the trial, which are side effects lasting over two days or requiring extra treatment. Examples include injury, illness, falls, joint pain, upper respiratory tract infections, sprains, strains, and muscle pain. Adherence reminder sessions will occur face-to-face, covering potential adverse events and appropriate actions, emphasizing contacting the clinic for relevant symptoms. Follow-up sessions will address participant concerns and reinforce key messages from the initial session. Significant AEs will be reported to chief investigators within one day. Participants experiencing AEs will cease intervention and be withdrawn from the study.

Randomization

An independent researcher will use computer-generated random sequencing to allocate participants in a 1:1:1:1:1:1 ratio via http://randomizer.org. Randomization will follow a blocked model with a block size of 24, concealed in numbered, sealed envelopes. Group allocation will be revealed to clinicians just before the first session. Participants will not be blinded due to the nature of the intervention.

Intervention

Licensed physical therapists experienced in managing PwMS will conduct interventions. Each group will have supervised sessions twice a week for 12 weeks, totaling 24 sessions. Each session will be approximately 60 minutes long, with regular breaks.

Water Environment

The pool depth is 120 cm, approximately aligned with the participants' xiphoid process level. Water temperature will be maintained at a constant 30–31°C, while the room temperature will be kept at 26–28°C (51).

Land environment

Electric coolers and cold neck packs will be provided to reduce the risk of hyperthermia and symptomatic fatigue during exercise.

Dual-task training

DTT will integrate motor tasks with cognitive tasks. In this study, DTT is founded on Veldkamp et al. with a few modifications (52). Motor activities will encompass walking at a preferred speed, fast walking, change in gait speed, backward walking, walking with a full cup of water, tandem walking, plantarflexion walking, and dorsiflexion walking, concurrently with cognitive tasks. Motor tasks are chosen based on prior research investigating reliability among individuals with neurological disorders engaging in various walking activities (43, 53, 54).

Cognitive functions addressed include fluency, working memory, selective attention, auditory discrimination, processing speed, executive function, text comprehension, sustained attention, auditory memory, visual discrimination, visual-spatial imagery tasks, and verbal and visual reasoning. All domains are selected due to their demonstrated early impairment in the PwMS (7, 8). During the session, participants will be allowed adequate rest between exercises to manage their fatigue. The DTT protocol is detailed in Table 2.

For the baseline, participants' steps will be tracked while performing various walking tasks using a pedometer. Following each DTT, their steps will be tracked again, and they will receive feedback on their performance based on answer accuracy and steps tracked. Participants will advance to a higher difficulty level if they maintain ≥70% of baseline steps per minute of DTT and achieve ≥70% accuracy in their responses. If the number of steps per minute of DTT falls below <50% of the baseline, participants will be instructed to return to a lower difficulty level. This instruction is necessary if the accuracy of responses also falls below <50%, or if it is deemed necessary by the practitioner for safety or quality reasons (55).

Table 2. Dual-task training

Motor function	**Exercise description / Difficulty levels**
Preferred walking speed	Walking with preferred speed for 2 min.
Fast walking speed	1. for 30 seconds. 2. for 60 seconds. 3. for 90 seconds.
Change in walking speed	1. Perform for 2 minutes with a 30-second interval, and an execution time of 10 seconds. 2. Perform for 2 minutes with a 20-second interval, and an execution time of 10 seconds. 3. Perform for 3 minutes with a 30-second interval, and an execution time of 10 seconds.
Backward walking	Walking with preferred speed for 1 min.
Walking with full cup of water	1. Walking with preferred speed for 1 minutes. 2. Walking with fast speed for 30 seconds.
Tandem walking	2. for 2 minutes. 3. for 1 minutes.
Plantarflexion walking	Walking for 30 seconds.
Dorsiflexion walking	Walking for 30 seconds.
Cognitive function	**Exercise description / Difficulty levels**
Working memory, **Verbal fluency**	Say a word to follow guidelines. 1. the word's first letter is given 2. the word's last letter is given. 3. the word's 2nd letter is given.
Working memory, **Selective attention,** **Auditory discrimination**	recognize words that are: 1. semantically different among other words. 2. from the same category. 3. from the same category or semantically different.
Working memory **Executive function**	1. Spell a 4- letter word backward. 2. Spell a 4 to 6, letter word backward.

	3. Spell a 7+ letter word in backward.
Working memory	reverse the order of stating numbers, days, or months (e.g. Wednesday, Tuesday, Monday, etc.).
sustained attention **processing speed**	subtract 3 repeatedly from a hundred. subtract 4 repeatedly from a hundred. subtract 7 repeatedly from a hundred.
working memory **processing speed** **verbal analog reasoning**	Combine language and computation “If Wednesday is the 15th, what will the date of next Saturday be?" "What day will it be in three days if it is the 15th of the month today?"
Verbal fluency **Executive function**	Explain a word, without 1. directly mentioning it. 2. utilizing derived vocabulary. 3. resorting to gestures. 4. refraining from using abbreviations or hints implying its sound.
Text comprehension **Verbal memory** **Sustained attention**	answer the questions after listening to the story: 1. 3 multiple-choice (3 options) questions about the story. 2. 3 multiple-choice (4 options) questions about the story.
Auditory memory (recognition)	Two series of words are read. Figure out if any word heard in the second sequence has been heard before or not.
executive function **Visual imaginary spatial task**	name the opposite direction of the actions. say the word “left” when they move their right hand.
Selective attention	Stroop task name the color of a printed word while ignoring the word's actual meaning e.g., the word "RED" written in blue ink is blue.
Auditory discrimination **Working memory** **Selective attention**	recognizing the sounds or voices from a compact disc 1. voices (man, woman, child). 2. sounds (bicycle bell, car start, dog bark, …).
Visual discrimination	Similarities and differences between the two images. 1. more than one difference (simple images). 2. one difference (difficult images). 3. more than one difference (difficult images).
Visual imaginary spatial task	Imagine the direction of the path (the route from their residence to the swimming pool or gym)
Verbal and visual analog reasoning	calculation assignment adding or subtracting from letters (e.g., M + 2= O).

Combined Exercise training

Prior to main training, there will be an initial warm-up that includes exercises targeting the lower and upper limbs (squats, lateral lunges, horizontal arm extension, and vertical arm extension using resistance bands). The protocol will follow the recommendations of Kim et al. (2019) and Latimer-Cheung et al. (2017) (56, 57), in which sets, repetitions, and loads (averaged based on the percentage of the 1 repetition maximum) are defined as light (up to 40%), moderate (between 40% and 60%), and high (up to 60–80%).

The macrocycle training will progress as follows: the first and second weeks will consist of 2 sets of 10 reps with a light load; this will transition to 2 sets of 10 reps with a light to moderate load in the third and fourth weeks; The fifth and sixth weeks will consist of 2 sets of 12 reps with a moderate load; Weeks seven and eight will consist of 3 sets of 10 repetitions with a moderate load; Weeks nine and ten will include 2 sets of 10 reps with a moderate to high load; and finally, weeks eleven and twelve will consist of 2 sets of 8 reps with a high load. The rest period between sets and muscle groups will be approximately 2 minutes.

Aerobic training involves 10 minutes of cycling on an ergometer, maintaining a heart rate (HRmax) of 40-70% according to the Karvonen formula: exercise HR = percentage of target intensity (HRmax - HRrest) + HRrest. Patients will utilize a heart rate monitor (Polar H9, Finland) during each session. Progression in intensity will be determined based on the tolerance of the PwMS. Patients will be encouraged to communicate their sensations during the exercise sessions, while the assistant will also observe their facial expressions for signs of discomfort to ensure the sessions' duration and frequency are well tolerated.

Balanced training will be conducted based on three balanced dimensions:

1. Stable base of support (BOS): Maintaining a stable base of support throughout the balance training by utilizing the center of mass. 2. Sway: Voluntarily shifting the center of mass to the limits of stability. 3. Step and walk: Creating and managing a new base of support by intentionally shifting the center of gravity beyond the stability limit.

Balance training will comprise 10-minute exercises, each lasting 15 seconds. These exercise training progress from easy to challenging, based on Sattelmeyer et al. (2021) (58). The protocol concludes with stretching and cooling down major muscle groups. For detailed protocol information, refer to Table 3.

Table 3. Combined exercise training

	Type	Frequency	Intensity	Time
Warm-up	low-intensity walking	2 days per week	Up to 30% HRpeak	5-10 min
Strength training	Squats, Lateral Lunges, Horizontal arm extension, Vertical arm extension using resistance bands.	2 days per week	light (up to 40%) medium (between 40% and 60%) high (up to 60–80%) 2-3 sets between 8–12 repetitions of each .	30-25 min
Aerobic training	Cycling on an ergometer	2 days per week	40–70% HRpeak	10 min
Balance training	Standing wide stance Standing feet together Step stance wide feet position Semi-tandem stance Stable Tandem stance feet apart Stable Tandem stance Stable One leg stance Stable Wall leaning forwards Wall leaning backwards Standing moving body sidewards Rolling ball forwards Stepping forwards Stepping sidewards Stepping backwards Leaning forward reactive step Line walking Walk backwards Heel walking Forefoot walking	2 days per week	2-3 balance exercises each session, Progressing from dimension 1 to dimension 3.	10 min each exercise for 15s
Stretch and cool down	Static stretching of major muscle groups	2 days per week	Stretch to the point of feeling tightness	5 min Hold 30-60s 2-3 repetition

Control interventions

This will include 60-minute maximum supervised cycling sessions on an ergometer. Participants are free to take a break whenever they desire, with an intensity set at 30–40% of their maximum heart rate. A great deal of PwMS is advised to utilize a cycle ergometer as it requires less balance and coordination than walking on a treadmill (59). Moreover, continuous walking for an hour increases the possibility of fatigue, monotony, and adverse events for PwMS. Therefore, selecting the cycle ergometer as a comparator is justified.

Assessment

Each participant will undergo evaluation by an independent, trained, blinded assessor who will be unaware of the group allocation. There will be two separate assessments utilizing approved and validated outcome measures: one at baseline and one after the intervention.

Demographic and diagnostic baseline data

All participants will have their age, gender, weight, height, BMI, level of education, number of years since the onset of symptoms, duration since diagnosis, the six-minute walk test (6MWT), the timed 5-repetition sit-to-stand, and Berg Balance Scale recorded on a standardized data collection form. In addition, data on the medications currently being taken and those taken over the research period will be acquired.

Outcomes

The Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) is a measurement tool that will be used to assess cognitive impairment. It will be administered in one session by a neurologist in a quiet room and typically takes about 15 minutes to complete. The BICAMS includes three tests: the Symbol Digit Modalities Test (SDMT), the California Verbal Learning Test-II (CVLT-II), and the Brief Visuospatial Memory Test-Revised (BVMT-R) (60, 61).

Primary outcomes

The Symbol Digit Modalities Test (SDMT)

The SDMT measures processing speed, which is the primary cognitive impairment in PwMS. Participants will receive a series of nine meaningless geometric symbols, each numbered one through nine and will be required to orally match these symbols with the corresponding numbers in the correct sequence within a ninety-second timeframe. The score equals the number of correct substitutions.

Secondary outcome

California Verbal Learning Test-II (CVLT-II)

The test will involve participants listening to a list of 16 words read by the examiner. They will then attempt to recall as many words as possible in any order. After the initial recall, the list will be read again, and participants will attempt to recall more words. This process will be repeated for five trials.

Brief Visuospatial Memory Test-Revised (BVMT-R)

Participants will view six abstract designs for 10 seconds each, then mark interpretations on paper. Each design earns 0 to 2 points based on accuracy and location, totaling 0 to 12 points. The test includes three trials, measuring performance by total points earned.

Nationality influence on BICAMS

Smerbeck et al. (62) examined the influence of nationality on BICAMS in PwMS across multiple countries including Iran. They found significant impacts on all three tests due to variations in test formats and socio-cultural contexts, including language effects and quality of translations. Factors such as educational backgrounds and linguistic nuances, such as familiarity with left-to-right languages, could influence performance. Therefore, when implementing BICAMS globally, including norm-setting, accounting for nationality is essential (62).

Kinematics and kinetics

Kinematic and kinetic data will be collected at 120 Hz using 6 Vicon MX T40-S cameras, Vicon Workstation software (Oxford Metrics Ltd., Oxford, UK), and 2 Kistler force plates (Kistler Instruments AG, Winterthur, Switzerland). Individual spherical 14 mm retro-reflective markers will be placed as follows: C7, T10, clavicle : On the jugular notch where the clavicles meet the sternum, Sternum: On the xiphoid process of the sternum, shoulder: On the acromio-clavicular joint, finger: Just proximal to the middle knuckle on the hand, anterior superior iliac spine (ASIS), posterior superior iliac spine (PSIS), left thigh: Over the lower lateral 1/3 surface of the thigh, right thigh: Over the upper lateral 1/3 surface of the right thigh, knee: On the Lateral Epicondyle of the knee, left tibia: Over the lower 1/3 surface of the shank, right tibia: Over the upper 1/3 surface of the right shank, ankle: On the lateral malleolus along an imaginary line that passes through the transmalleolar axis, heel: On the calcaneus at the same height above the plantar surface of the foot as the toe marker, toe: Over the second metatarsal head, on the mid-foot side of the equinus break between the forefoot and mid-foot, for both the left and right sides.

The Vicon Workstation software will be utilized for data processing, encompassing joint kinematics and kinetics calculations, with code generated by Vicon Bodybuilder. Kinetic data will be normalized relative to body mass. Essential spatiotemporal parameters (stride time, cadence, gait speed, and stride length), alongside measures of relative phase (stance phase, swing phase, double stance phase, single support), will be extracted from all systems. Sagittal plane angles and moments at the hip, knee, and ankle throughout the gait cycle will be evaluated. Additionally, anteroposterior, mediolateral, and vertical ground reaction forces will be assessed. All testing sessions will entail patients being barefoot without socks.

Walk

Participants will receive instructions to walk a distance of 6 meters across the force plate at their preferred speed, after familiarizing themselves with the markers. Each leg will undergo three trials, ensuring a successful strike on the force plate each time, which will then be averaged.

Balance

Two methods will be employed to assess static balance: Eyes Open Feet Apart (EOFA) and Eyes Open Feet Together (EOFT). Participants will be instructed to maintain stillness for three trials under each condition, each lasting 20 seconds.

The dynamic balance will be evaluated through three rounds each of the Functional Reach Test (FRT) and Lateral Reach Test (LRT) on both dominant and non-dominant sides. Average scores will be calculated from these trials for each test, and scores will be adjusted based on participant height for inter-individual comparisons.

Data management

The digital recruiting list will be securely stored and password-protected separately from participant identifiers. Access to this list will be restricted to the chief investigators. Registration records for MS will also be securely saved and password-protected. Personally identifiable information will only be used for confirming duplicate entries, after which names and phone numbers will be removed, leaving unique identification numbers. Interview participants will not be asked for personal information. Consent forms will be accessible only to the chief investigators, and stored securely in a locked cabinet. Personally identifiable information will never be shared, and results will be presented only in aggregate form. Study data will be accessible solely to the chief investigators.

Sample Size

Our power calculations are based on Borland et al. (63), who identified the MCID of SDMT for mild cognitive impairment. Borland et al. reported the MCID as -3.8 and the effect size as -0.3 for minimal change ≥0.5 in the Clinical Dementia Rating—Sum of Boxes for the SDMT. Based on Borland et al., and considering a significance level of 0.05 and 90% power, we will need a total of 190 participants using G*Power software. Assuming a dropout rate of around 20%, we will need

228 participants. Regardless of dropouts, we will conduct intention-to-treat analyses for all initially assigned patients. This involves considering all participants in the analysis, regardless of whether they complete the study.

Statistical analysis

Statistical analysis will be performed using SPSS version 23.0. Demographic characteristics, study characteristics, and baseline data will be summarized using descriptive statistics. The Statistical methods to be used for analyzing the study include two-way analysis of variance (ANOVA) to assess the main environmental effects (land and water), the effects of training conditions (DTT, combined exercise training, controlled), and any possible interaction effects between environmental and training conditions. Additionally, repeated measures ANOVA will be used to compare pre-post assessments within groups (land and water) and conditions (DTT, combined exercise training, control). The results will be reported according to the CONSORT Statement (2010) for the reporting of multi-arm factorial trials (64).

Ethics and dissemination

This study was approved by the Sport Sciences Research Institute (SSRI) (No. IR.SSRC.REC.1401.082) and adheres to the Declaration of Helsinki. Any protocol changes will be communicated to ethics boards and participating districts. Patients will sign informed consent forms. Results will be published in peer-reviewed journals and presented at MS conferences. Additional information will be available from the corresponding author post-publication.

Discussion

Although MS is not very common, its prevalence has significantly increased in recent years, especially since 2013 (9). In Iran, the percentage change in age-standardized prevalence rates was reported as 40.4% between 1990 and 2016; however, during this period, the global percentage change in age-standardized prevalence rates was reported as 10.4% (65). This underscores the importance of raising awareness and managing MS disease both nationally and globally. Exercise training is a valuable secondary strategy alongside pharmacological treatment. One notable exercise training method is "multidisciplinary rehabilitation" which combines exercise with non-exercise treatments, such as pairing medication with training exercises, performing exercises alongside cognitive activities (dual-tasking), or combining different types of exercise like endurance, strength, and balance exercises (combined exercise training) (16, 66). Multidisciplinary rehabilitation acknowledges that a singular treatment is insufficient for addressing the varied symptoms of MS. It is likely that current interventions operate through separate pathways or mechanisms without overlap (16). Combining exercise training with DTT provides a more precise representation of the complex demands of daily activities. These tasks replicate the challenges encountered during everyday tasks like driving, gardening, vacuuming, and walking pets, where individuals must perform cognitive and motor tasks simultaneously. Studies showed that both DTT and combined exercise training improved cognitive function, motor performance, walking capacity, walking speed, and dynamic balance (17, 22, 37, 38).

These interventions (combined and dual-tasks) can be performed in both water and land environments. Several studies have examined how aquatic interventions affect PwMS (24-27). A meta-analysis found that aquatic training benefits both physical and mental well-being. This is particularly important for PwMS, who often experience depression and anxiety (31).

As far as we examined, no studies were studied the effects of cognitive and motor factors (kinetics and kinematics of walking and balance) on DTT and combined exercise training in aquatic and land environments. As the prevalence of MS is increasing, heightened awareness may provide researchers and patients with a broader perspective on therapeutic exercises and effective symptom management in the future. In addition, these findings can help healthcare professionals prescribe and develop more effective exercise programs for PwMS. Furthermore, this study addresses important evidence gaps and provides clinical insights for MS management decision-making.

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Authors Contributions

S.N., S.S., and M.K. contributed to the original idea, study design, protocol, conception of the work, conducting the study, and undertook the sample size calculations. S.N., S.S. standardized the data collection forms contributed to the conception of the work, writing, and editing of this article. M.K. will be involved in compiling and analyzing the data. M.K. will contribute to data interpretation. All the authors participated in critical revisions of the manuscript and read and approved the final manuscript.

The Alborz MS NGO will participate in the recruitment of patients based on the inclusion criteria. In order to evaluate adverse events and side effects, this association will participate. Also, cognitive pre and post-tests will be performed by neurologists of the Alborz MS Association.

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. further information will be accessible from the corresponding author after the completion of the trial.

Competing interest's statement

The study design, implementation, management, analysis, interpretation, and reporting of the study are entirely independent of the Kharazmi University and The Alborz MS NGO. The authors declare no conflicts of interest.

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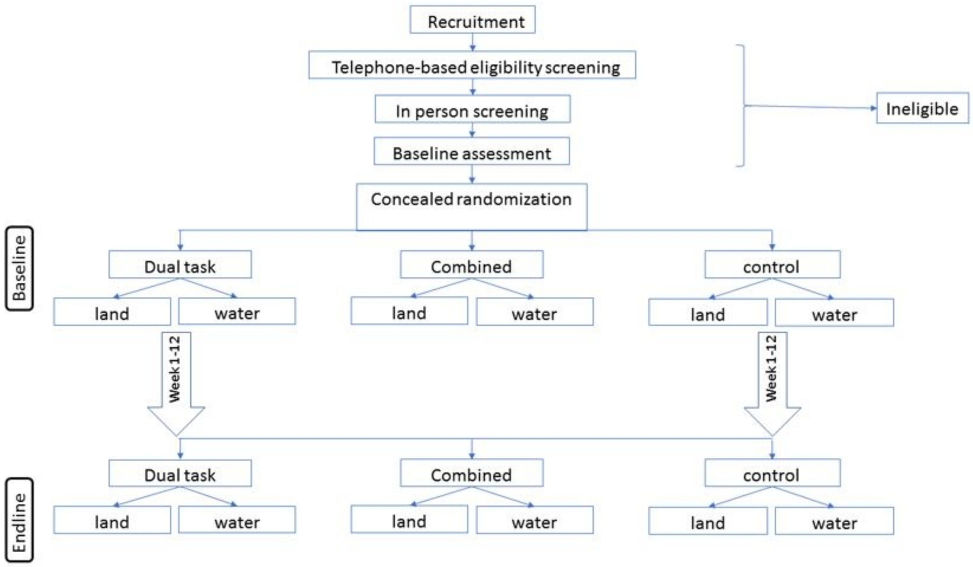

fig 1. **Trial profile.** combined, dual-task or control group, with either in water or on land at baseline and endline

fig. 1. trial profile. Combined, dual-task and control group, either in water or on land at baseline and endline.

178x121mm (120 x 120 DPI)

Intervention type	environment	Before and after 12 weeks	
combined	In water	Baseline	Endline
		Baseline	Endline
	On land	Baseline	Endline
		Baseline	Endline
Dual task	In water	Baseline	Endline
		Baseline	Endline
	On land	Baseline	Endline
		Baseline	Endline
control	In water	Baseline	Endline
		Baseline	Endline
	On land	Baseline	Endline
		Baseline	Endline

Fig 2. Intervention training protocol. The 2 × 2 × 3 factorial design, showing the 12 Intervention training protocol based on combined, dual task or control group, with either in water or on land and baseline and endline.

fig. 2. Intervention training protocol. The 2*2*3 factorial design, showing the 6 intervention training protocol, based on combined, dual-task, and control group, either in water or on land at baseline and endline.

106x147mm (120 x 120 DPI)

BMJ Open

Comparative effects of dual-task training versus combined exercise training in water and on land on patients with multiple sclerosis: A Study protocol of a randomized factorial trial

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Comparative effects of dual-task training versus combined exercise training in water and on land on patients with multiple sclerosis: A Study protocol of a randomized factorial trial

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Abstract

Introduction: People with multiple sclerosis (PwMS) experience cognitive and motor impairments, including cognitive training to exercise training. This study compares dual-task and combined exercise training in water and on land. Water-based training may enhance cognitive and motor function more effectively than land-based training, presenting a promising intervention for PwMS. We explore dual-task training including cognitive and motor exercise with combined exercise in water and on land on the cognition, balance, and gait PwMS.

Methods and analysis: This is a double-blind 3*2*2 factorial randomized trial. Participants will be randomly allocated to one of six groups: four intervention groups and two control groups. All patients will receive supervised 12-week training sessions, twice a week, and will be assessed by a blinded outcome assessor before and at the end of the 12-week program. The primary outcome includes The Symbol Digit Modalities Test (SDMT). The secondary outcomes involve the California Verbal Learning Test-II (CVLT-II), the Brief Visuospatial Memory Test-Revised (BVMT-R), kinetics, and kinematics throughout the balance and gait cycle. All the data will be analyzed by a blinded data analyst.

Ethics and dissemination: Ethical approval was granted by the Sports Science Research Institute (SSRI) (No. IR.SSRC.REC.1401.082). The results of the trial will be submitted for publication in journals and distributed to PwMS and physiotherapists.

Trial registration number: The trial is prospectively registered on 2024/03/22, at https://www.umin.ac.jp/ with an identification number (UMIN000053947).

Keywords: Dual-task training, Combined exercise training; Multiple sclerosis; Cognition; Gait; Balance.

Strengths and limitations of this study

- Combined and dual-task exercise training is conducted in both water and land settings, allowing for a comparative analysis of environmental effects.
- Cognitive impairments and physical activity will be assessed using established cognitive assessment tools and comprehensive kinetics and kinematics measurements.
- While outcome assessors are blinded, participants and physical therapists will not be blinded to group allocation, which may introduce bias.
- The intervention follows a detailed, standardized protocol designed to ensure consistency across all training sessions.
- The study incorporates a robust system for monitoring adverse events, ensuring participant safety throughout the trial.

Introduction

Multiple sclerosis (MS) is a neurologic disease that adversely affects the central nervous system (CNS) through inflammation and demyelination. More than 2.8 million people worldwide are affected by MS, with a higher prevalence among women than men [1]. One of the most common phenotypes of MS is relapsing-remitting (RRMS) [2]. RRMS is typically diagnosed when PwMS have lesions in their nervous system or experience intermittent episodes of neurological symptoms involving inflammation over time [3], leading to demyelination, axon injury, loss of nerve cells, and subsequent atrophy [4]. Hence, PwMS often experiences a range of symptoms, including cognitive issues and motor dysfunctions. Studies investigated that 40-65% of PwMS and 30-45% of individuals with RRMS exhibit cognitive impairment [5, 6]. Common symptoms of cognitive impairment include decreased processing speed, deficits in learning and memory, perceptual skills, executive functions, and rarely linguistic or intellectual impairment [7]. Cognitive domains are crucial for executing motor tasks smoothly and managing complex actions. For instance, processing speed is vital for efficient signal transmission between the CNS and motor movement. Additionally, it predicts performance in executive functions and daily activities [8]. Executive functioning encompasses cognitive skills required for engaging in complex, goal-directed behavior and adapting to environmental demands, including abilities like planning and anticipating outcomes [8]. Therefore, deficits in cognitive domains can significantly impair motor function and the performance of daily activities in PwMS.

Due to the interaction between decreased physical and cognitive abilities, up to 75% of PwMS experience issues with balance and gait, both in the early stages and advanced stages of the disease [9]. Balance problems can also result from CNS damage or abnormal gait, leading to falls, injuries, loss of mobility, and reduced quality of life [10]. Moreover, there is evidence that balance depends not only on the integration of somatosensory, visual, and vestibular information but also on higher brain systems responsible for the memory needed for anticipatory movement [11].

Several studies have highlighted the walking difficulties in PwMS; Benedetti et al. reported that PwMS had a slower progression pace, shorter steps, and prolonged double support intervals during walking [12]. Coca-Tapia et al. showed a decrease in speed, step lengths, and stride lengths, alongside increased step width. They also noted decreases in hip extension during the stance phase, reduced knee flexion during the swing phase, decreased ankle dorsiflexion at initial contact, and decreased ankle plantar flexion during the preswing phase [13].

The decline in cognitive and physical performance significantly affects the daily activities and independence of PwMS [14], underscoring the critical role of symptom management. Managing symptoms is fundamental for promoting health and well-being in PwMS [15]. Exercise training is a valuable complement to disease-modifying drugs for managing MS and its symptoms [16], highlighting the need for effective non-pharmacological treatments like exercise therapy.

Unlike single-motor tasks, which may not fully represent daily activities, dual-tasks better simulate the cognitive and motor challenges of daily life [17]. Therefore, evaluating dual-task performance and tracking improvements can provide valuable insights into the overall functional status of PwMS. For instance, Kessler et al. found that difficulties in new learning significantly impacted activities of daily living, including shopping, housework, and transportation [18]. Additionally, safe functional movements necessitate simultaneous motor and cognitive tasks which are referred to as dual-task [19]. PwMS exhibit a reduced ability to perform dual-tasks [20], even in those with low EDSS scores [21]. It has been demonstrated that DTT has a moderately positive impact on enhancing dynamic balance and functional mobility in PwMS [22]. Additionally, it can improve gait speed, motor performance, and cognitive function in PwMS [17, 23].

Previous research indicates that aquatic training confers notable advantages over land-based training in MS research, notably due to buoyancy, viscosity, and thermodynamics [24]. PwMS commonly experience pain and reduced mobility [24]. Buoyancy mitigates joint impact, facilitating movements that may be challenging on land [25]. Also, Buoyancy mitigates muscle weakness and reduces the risk of fall-related injuries and fractures, prevalent among PwMS [26]. Studies have demonstrated that participating in aquatic activities can enhance muscle strength and reduce fatigue, both common concerns for PwMS [24, 27]. Viscosity provides drag forces, allowing multi-directional performance and offering low-impact resistance training, which can improve muscle strength and endurance [24, 27]. Land-based training also improves resistance; however, it can be adjusted by using weights or resistance bands.

Cerebral blood flow issues in PwMS lead to cognitive impairment, lesion formation, axonal degeneration, and fatigue [28]. Immersing in water may enhance cognitive function by promoting cerebral blood flow through hydrostatic pressure, stimulating mechanoreceptors, and increasing parasympathetic activity, which may positively influence attention and processing speed [29, 30]. Thermodynamics is advantageous due to the Uhthoff phenomenon, wherein MS symptoms exacerbate with increasing temperatures [31]. Water conducts heat 25 times faster than air, leading to more efficient regulation of body temperature during aquatic training compared to land-based exercises, due to water's superior heat retention capacity and faster heat transfer properties [24]. Previous studies have demonstrated the benefits of various cooling strategies before and after exercise training for PwMS [24, 32]. Among these strategies, engaging in aquatic exercise training in a cool pool stands out as an effective method to mitigate heat sensitivity.

Multiple studies have investigated the effectiveness of various exercise training interventions on in PwMS [26, 27, 33, 34]. However, a notable limitation is the tendency to focus on a singular mode of exercise training, neglecting the multifaceted physiological decline (e.g., aerobic deconditioning, muscle weakness, and balance dysfunction) in PwMS [35]. In addition to the previous emphasis on addressing balance deficiencies in PwMS [10], there is evidence suggesting that PwMS also experience decreases in both muscular and cardiorespiratory fitness levels. Lambert et al. found that PwMS exhibited significantly lower peak torque in various leg muscles compared to healthy individuals [33]. Similarly, Moatert et al. observed that cardiorespiratory fitness, measured by VO2peak, was 28% higher in healthy controls than in PwMS [34]. The decreasing fitness levels in PwMS, coupled with the vital importance of physical fitness for improving functionality and managing symptoms, highlight the critical need for concurrent interventions improving balance, gait, muscle strength, cardiovascular health, and cognitive functions [36-38]. Accordingly, Sandroff et al. [36] demonstrated that engaging in multimodal exercise training could enhance endurance walking performance and cognitive processing speed in PwMS experiencing significant mobility limitations. This improvement is likely attributed to enhancements in cardiorespiratory capacity. Motl et al. linked aerobic fitness with increased volumes of subcortical grey matter structures such as the hippocampus and basal ganglia [39], possibly explaining exercise's positive impact on ambulation and cognition [40]. We will therefore conduct a 12-week factorial trial to evaluate the effect of DTT and combined exercise training in water and land, on cognition, balance, and gait in PwMS.

Aims and hypothesis

The primary objective of this study is to compare the effects of DTT and combined exercise training in water and on land on cognitive impairment in PwMS and to examine the interaction effects of these treatments. Given that processing speed decline is central to cognitive impairment, it will be a key outcome to assess intervention effectiveness. Evaluating the effects of these interventions on gait kinetics and kinematics, including spatiotemporal parameters, sagittal plane angles, and moments of the hip, knee, and ankle, as well as anteroposterior, mediolateral, and vertical ground reaction forces in two different environmental conditions, remains secondary. The hypothesis posits that aquatic environments may enhance cognition and motor function more effectively than land environments. This is likely due to increased blood flow and mechanoreceptor stimulation during water immersion, which may help reduce cognitive impairment and enhance motor function. Another hypothesis suggests that combined exercise training may have a greater effect on gait kinetics compared to DTT. This is based on the importance of balance enhancement for improving gait, proprioception, coordination, and postural stability. Strengthening muscles is crucial for maintaining alignment and restoring the ability to respond quickly to stimuli, which can positively affect gait dynamics. Furthermore, aerobic exercises can enhance cardiovascular health, potentially increasing endurance during walking.

Method and Analysis

Study Design

This is a 2*2*3 factor randomized study with double-blind outcome assessor and the data analyst will be conducted at the campus of Kharazmi University, Tehran, Iran. The study is scheduled to begin on April 6, 2024, and is expected to conclude on July 29, 2024.

The trial was registered with the UMIN Clinical Trials Registry due to technical issues with the Iranian clinical trial registry at the time of registration. The decision to use UMIN was based on its availability and functionality. It is important to note that, although the registration occurred on a foreign platform, the trial is being conducted in Iran, following all national ethical and regulatory requirements, including ethical approval from the Sports Science Research Institute (SSRI) (No. IR.SSRC.REC.1401.082). Additionally, we had previous experience with UMIN from a prior study, which further influenced this choice.

The protocol is described according to Recommendations for Interventional Trials (SPIRIT) guidelines (Table 1), and the results of the study will be presented in a CONSORT statement (Fig. 1).

Table 1. SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) diagram of enrolment, interventions, and assessments of the trial.

	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
TIMEPOINT	$-t_1$	0	Week 4	Week 8	Week 12	t_x
Enrolment						
Eligibility screen	X					
Informed consent	X					
Cognitive impairments assessment	X					
Safety for exercise clearance	X					
Allocation		X				
Interventions						
Dual-task in water						

Dual-task on land						
Combined in water						
Combined on land						
Control in water						
Control on land						
Clinical measures						
Weight, Height, BMI		X				
Level of education		X				
Symptoms duration		X				
Diagnosis duration		X				
Medication use		X				
Assessment						
BICAMS		X				X
Gait characteristics		X				X
Balance assessment		X				X
Safety (AEs)	X	X	X	X	X	X
Early withdrawal information	As required					
AEs: adverse events, BICAMS: Brief International Cognitive Assessment for MS						

Patient and public involvement

The study is crafted through extensive cooperation among PwMS, healthcare experts, and researchers. Additionally, representatives from the Alborz MS NGO of Iran, who are also PwMS, are involved as project partners to guarantee the benefits for patients.

Eligibility criteria

Participants are eligible to be included in the trial if they meet the following inclusion criteria: PwMS will be aged between 20 and 60, including both males and females, diagnosed with relapsing-remitting MS as outlined by the McDonald criteria [41], the EDSS scores ranging between 1.0 and 5.5 [42], Relapse-free for the last 3 months [43], have not engaged in regular physical exercise (no training within the last three months), be willing to participate in the current study, Cognitive impairments that will be characterized by scores below the standard criterion of at least 1.5 standard deviations [8] on at least one assessments from the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) [44].

Potential participants will be excluded if they are unable to comply with the requirements of the protocol, inability to stand and walk without aids and braces, changes in medication in the past 2 months [45], history of cardiovascular, kidney, or other chronic diseases, pregnancy in the past 12 months [45], medical conditions interfering with mobility, MS-like syndromes such as neuromyelitis optica, or major problems with hearing, vision, and perception [46].

Recruitment

We will recruit PwMS from the Alborz MS NGO of Iran. Community-based recruitment will be conducted through a multi-channel approach, involving collaboration with PwMS themselves, referrals from neurologists, and advertisements via both traditional (e.g., hospital boards, printed flyers) and digital platforms (e.g., Instagram, social media groups). Based on the inclusion criteria, the researchers will enroll eligible PwMS in the study. At enrollment, contributors will be randomly assigned to one of six groups: dual-task training in water, dual-task training on land, combined exercise training in water, combined exercise training on land, control group in water, and control group on land (Fig. 2).

Screening

Volunteers will undergo a telephone assessment to determine eligibility. Those meeting the criteria will be invited for a screening appointment at the trial center. This includes MS EDSS evaluation, medication history questionnaires, exercise safety clearance, assessment of baseline physical activity, and evaluation for cognitive impairment by a neurologist.

Safety for Exercise Clearance

A pre-exercise assessment will identify PwMS at higher risk of adverse events during exercise. Functional assessments will be utilized to develop exercise program recommendations, aligned with American Sports Medicine Association guidelines [47]. These include the six-minute walk test (6MWT), where patients walk as fast as they comfortably can for 6 minutes without running or jogging, aimed at measuring functional endurance [48]. The timed 5-repetition sit-to-stand assesses the time taken to complete five repetitions of the sit-to-stand movement as quickly as possible, serving as a measure of strength [49]. Furthermore, the Berg Balance Scale evaluates patients' performance on 14 items, comprising 5 static and 9 dynamic items, related to balance function frequently encountered in everyday life [50].

Safety

We will monitor adverse events (AEs) during the trial, which are side effects lasting over two days or requiring extra treatment. Examples include injury, illness, falls, joint pain, upper respiratory tract infections, sprains, strains, and muscle pain. Adherence reminder sessions will occur face-to-face, covering potential adverse events and appropriate actions, emphasizing contacting the clinic for relevant symptoms. Follow-up sessions will address participant concerns and reinforce key messages from the initial session. Significant AEs will be reported to chief investigators within one day. Participants experiencing AEs will cease intervention and be withdrawn from the study.

Randomization

An independent researcher will use computer-generated random sequencing to allocate participants in a 1:1:1:1:1:1 ratio via http://randomizer.org. Randomization will follow a blocked model with a block size of 24, concealed in numbered, sealed envelopes. Group allocation will be revealed to clinicians just before the first session. Participants will not be blinded due to the nature of the intervention.

Intervention

Licensed physical therapists experienced in managing PwMS will conduct interventions. Each group will have supervised sessions twice a week for 12 weeks, totaling 24 sessions. Each session will be approximately 60 minutes long, with regular breaks. All interventions, including dual-task training, combined exercise training, and the control intervention, will be applied in both environments: land and water.

Water Environment

The pool depth is 120 cm, approximately aligned with the participants' xiphoid process level. Water temperature will be maintained at a constant 30–31°C, while the room temperature will be kept at 26–28°C [51].

Land environment

Electric coolers and cold neck packs will be provided to reduce the risk of hyperthermia and symptomatic fatigue during exercise.

Dual-task training

DTT will integrate motor tasks with cognitive tasks. In this study, DTT is founded on Veldkamp et al. with a few modifications [52]. Motor activities will encompass walking at a preferred speed, fast walking, change in gait speed, backward walking, walking with a full cup of water, tandem walking, plantarflexion walking, and dorsiflexion walking, concurrently with cognitive tasks. Motor tasks are chosen based on prior research investigating reliability among individuals with neurological disorders engaging in various walking activities [43, 53, 54]. Cognitive functions addressed include fluency, working memory, selective attention, auditory discrimination, processing speed, executive function, text comprehension, sustained attention, auditory memory, visual discrimination, visual-spatial imagery tasks, and verbal and visual reasoning. All domains are selected due to their demonstrated early impairment in the PwMS [7, 8]. During the session, participants will be allowed adequate rest between exercises to manage their fatigue. The DTT protocol is detailed in Table 2.

For the baseline, participants' steps will be tracked while performing various walking tasks using a pedometer. Following each DTT, their steps will be tracked again, and they will receive feedback on their performance based on answer accuracy and steps tracked. Participants will advance to a higher difficulty level if they maintain ≥70% of baseline steps per minute of DTT and achieve ≥70% accuracy in their responses. If the number of steps per minute of DTT falls below <50% of the baseline, participants will be instructed to return to a lower difficulty level. This instruction is necessary if the accuracy of responses also falls below <50%, or if it is deemed necessary by the

practitioner for safety or quality reasons [55].

Table 2. Dual-task training

Motor function	**Exercise description / Difficulty levels**
Preferred walking speed	Walking with preferred speed for 2 min.
Fast walking speed	1. for 30 seconds. 2. for 60 seconds. 3. for 90 seconds.
Change in walking speed	1. Perform for 2 minutes with a 30-second interval, and an execution time of 10 seconds. 2. Perform for 2 minutes with a 20-second interval, and an execution time of 10 seconds. 3. Perform for 3 minutes with a 30-second interval, and an execution time of 10 seconds.
Backward walking	Walking with preferred speed for 1 min.
Walking with full cup of water	1. Walking with preferred speed for 1 minutes. 2. Walking with fast speed for 30 seconds.
Tandem walking	2. for 2 minutes. 3. for 1 minutes.
Plantarflexion walking	Walking for 30 seconds.
Dorsiflexion walking	Walking for 30 seconds.
Cognitive function	**Exercise description / Difficulty levels**
Working memory, Verbal fluency	Say a word to follow guidelines. 1. the word's first letter is given 2. the word's last letter is given. 3. the word's 2nd letter is given.
Working memory, Selective attention, Auditory discrimination	recognize words that are: 1. semantically different among other words. 2. from the same category. 3. from the same category or semantically different.
Working memory Executive function	1. Spell a 4- letter word backward. 2. Spell a 4 to 6, letter word backward. 3. Spell a 7+ letter word in backward.
Working memory	reverse the order of stating numbers, days, or months (e.g. Wednesday, Tuesday, Monday, etc.).
sustained attention processing speed	subtract 3 repeatedly from a hundred. subtract 4 repeatedly from a hundred. subtract 7 repeatedly from a hundred.

working memory **processing speed** **verbal analog reasoning**	Combine language and computation “If Wednesday is the 15th, what will the date of next Saturday be?" "What day will it be in three days if it is the 15th of the month today?"
Verbal fluency **Executive function**	Explain a word, without 1. directly mentioning it. 2. utilizing derived vocabulary. 3. resorting to gestures. 4. refraining from using abbreviations or hints implying its sound.
Text comprehension **Verbal memory** **Sustained attention**	answer the questions after listening to the story: 1. 3 multiple-choice (3 options) questions about the story. 2. 3 multiple-choice (4 options) questions about the story.
Auditory memory (recognition)	Two series of words are read. Figure out if any word heard in the second sequence has been heard before or not.
executive function **Visual imaginary spatial task**	name the opposite direction of the actions. say the word “left” when they move their right hand.
Selective attention	Stroop task name the color of a printed word while ignoring the word's actual meaning e.g., the word "RED" written in blue ink is blue.
Auditory discrimination **Working memory** **Selective attention**	recognizing the sounds or voices from a compact disc 1. voices (man, woman, child). 2. sounds (bicycle bell, car start, dog bark, …).
Visual discrimination	Similarities and differences between the two images. 1. more than one difference (simple images). 2. one difference (difficult images). 3. more than one difference (difficult images).
Visual imaginary spatial task	Imagine the direction of the path (the route from their residence to the swimming pool or gym)
Verbal and visual analog reasoning	calculation assignment adding or subtracting from letters (e.g., M + 2= O).

Combined Exercise training

Prior to main training, there will be an initial warm-up that includes exercises targeting the lower and upper limbs (squats, lateral lunges, horizontal arm extension, and vertical arm extension using resistance bands). The protocol will follow the recommendations of Kim et al. (2019) and Latimer-Cheung et al. (2017) [56, 57], in which sets, repetitions, and loads (averaged based on the percentage of the 1 repetition maximum) are defined as light (up to 40%), moderate (between 40% and 60%), and high (up to 60–80%).

The macrocycle training will progress as follows: the first and second weeks will consist of 2 sets of 10 reps with a light load; this will transition to 2 sets of 10 reps with a light to moderate load in the third and fourth weeks; The fifth and sixth weeks will consist of 2 sets of 12 reps with a moderate load; Weeks seven and eight will consist of 3 sets of 10 repetitions with a moderate load; Weeks nine and ten will include 2 sets of 10 reps with a moderate to high load;

and finally, weeks eleven and twelve will consist of 2 sets of 8 reps with a high load. The rest period between sets and muscle groups will be approximately 2 minutes.

Aerobic training involves 10 minutes of cycling on an ergometer, maintaining a heart rate (HRmax) of 40-70% according to the Karvonen formula: exercise HR = percentage of target intensity (HRmax - HRrest) + HRrest. Patients will utilize a heart rate monitor (Polar H9, Finland) during each session. Progression in intensity will be determined based on the tolerance of the PwMS. Patients will be encouraged to communicate their sensations during the exercise sessions, while the assistant will also observe their facial expressions for signs of discomfort to ensure the sessions' duration and frequency are well tolerated.

Balanced training will be conducted based on three balanced dimensions:

1. Stable base of support (BOS): Maintaining a stable base of support throughout the balance training by utilizing the center of mass. 2. Sway: Voluntarily shifting the center of mass to the limits of stability. 3. Step and walk: Creating and managing a new base of support by intentionally shifting the center of gravity beyond the stability limit.

Balance training will comprise 10-minute exercises, each lasting 15 seconds. These exercise training progress from easy to challenging, based on Sattelmeyer et al. (2021) [58]. The protocol concludes with stretching and cooling down major muscle groups. For detailed protocol information, refer to Table 3.

Table 3. Combined exercise training

	Type	Frequency	Intensity	Time
Warm-up	low-intensity walking	2 days per week	Up to 30% HRpeak	5-10 mi
Strength training	Squats, Lateral Lunges, Horizontal arm extension, Vertical arm extension using resistance bands.	2 days per week	light (up to 40%) medium (between 40% and 60%) high (up to 60–80%) 2-3 sets between 8–12 repetitions of each .	30-2 mi
Aerobic training	Cycling on an ergometer	2 days per week	40–70% HRpeak	10 min

Balance training	Standing wide stance Standing feet together Step stance wide feet position Semi-tandem stance Stable Tandem stance feet apart Stable Tandem stance Stable One leg stance Stable Wall leaning forwards Wall leaning backwards Standing moving body sidewards Rolling ball forwards Stepping forwards Stepping sidewards Stepping backwards Leaning forward reactive step Line walking Walk backwards Heel walking Forefoot walking	2 days per week	2-3 balance exercises each session, Progressing from dimension 1 to dimension 3.	10 min each exercise
Stretch and cool down	Static stretching of major muscle groups	2 days per week	Stretch to the point of feeling tightness	5 min Hold 30-6 2-3 repetit

Control interventions

This will include 60-minute maximum supervised cycling sessions on an ergometer. Participants are free to take a break whenever they desire, with an intensity set at 30–40% of their maximum heart rate. A great deal of PwMS is advised to utilize a cycle ergometer as it requires less balance and coordination than walking on a treadmill [59]. Moreover, continuous walking for an hour increases the possibility of fatigue, monotony, and adverse events for PwMS. Therefore, selecting the cycle ergometer as a comparator is justified.

Assessment

Each participant will undergo evaluation by an independent, trained, blinded assessor who will be unaware of the group allocation. There will be two separate assessments utilizing approved and validated outcome measures: one at baseline and one after the intervention.

Demographic and diagnostic baseline data

All participants will have their age, gender, weight, height, BMI, level of education, number of years since the onset of symptoms, duration since diagnosis, the six-minute walk test (6MWT), the timed 5-repetition sit-to-stand, and Berg Balance Scale recorded on a standardized data collection form. In addition, data on the medications currently being taken and those taken over the research period will be acquired.

Outcomes

The Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) is a measurement tool that will be used to assess cognitive impairment. It will be administered in one session by a neurologist in a quiet room and typically takes about 15 minutes to complete. The BICAMS includes three tests: the Symbol Digit Modalities Test (SDMT), the California Verbal Learning Test-II (CVLT-II), and the Brief Visuospatial Memory Test-Revised (BVMT-R) [60, 61].

Primary outcomes

The Symbol Digit Modalities Test (SDMT)

The SDMT measures processing speed, which is the primary cognitive impairment in PwMS. Participants will receive a series of nine meaningless geometric symbols, each numbered one through nine and will be required to orally match these symbols with the corresponding numbers in the correct sequence within a ninety-second timeframe. The score equals the number of correct substitutions.

Secondary outcome

California Verbal Learning Test-II (CVLT-II)

The test will involve participants listening to a list of 16 words read by the examiner. They will then attempt to recall as many words as possible in any order. After the initial recall, the list will be read again, and participants will attempt to recall more words. This process will be repeated for five trials.

Brief Visuospatial Memory Test-Revised (BVMT-R)

Participants will view six abstract designs for 10 seconds each, then mark interpretations on paper. Each design earns 0 to 2 points based on accuracy and location, totaling 0 to 12 points. The test includes three trials, measuring performance by total points earned.

Nationality influence on BICAMS

Smerbeck et al. [62] examined the influence of nationality on BICAMS in PwMS across multiple countries including Iran. They found significant impacts on all three tests due to variations in test formats and socio-cultural contexts, including language effects and quality of translations. Factors such as educational backgrounds and linguistic nuances, such as familiarity with left-to-right languages, could influence performance. Therefore, when implementing BICAMS globally, including norm-setting, accounting for nationality is essential [62].

Kinematics and kinetics

Kinematic and kinetic data will be collected at 120 Hz using 6 Vicon MX T40-S cameras, Vicon Workstation software (Oxford Metrics Ltd., Oxford, UK), and 2 Kistler force plates (Kistler Instruments AG, Winterthur, Switzerland). Individual spherical 14 mm retro-reflective markers will be placed as follows: C7, T10, clavicle : On the jugular notch where the clavicles meet the sternum, Sternum: On the xiphoid process of the sternum, shoulder: On the acromio-clavicular joint, finger: Just proximal to the middle knuckle on the hand, anterior superior iliac spine (ASIS), posterior superior iliac spine (PSIS), left thigh: Over the lower lateral 1/3 surface of the thigh, right thigh: Over the upper lateral 1/3 surface of the right thigh, knee: On the Lateral Epicondyle of the knee, left tibia: Over the lower 1/3 surface of the shank, right tibia: Over the upper 1/3 surface of the right shank, ankle: On the lateral malleolus along an imaginary line that passes through the transmalleolar axis, heel: On the calcaneus at the same height above the plantar surface of the foot as the toe marker, toe: Over the second metatarsal head, on the mid-foot side of the equinus break between the forefoot and mid-foot, for both the left and right sides.

The Vicon Workstation software will be utilized for data processing, encompassing joint kinematics and kinetics calculations, with code generated by Vicon Bodybuilder. Kinetic data will be normalized relative to body mass. Essential spatiotemporal parameters (stride time, cadence, gait speed, and stride length), alongside measures of relative phase (stance phase, swing phase, double stance phase, single support), will be extracted from all systems. Sagittal plane angles and moments at the hip, knee, and ankle throughout the gait cycle will be evaluated. Additionally, anteroposterior, mediolateral, and vertical ground reaction forces will be assessed. All testing sessions will entail patients being barefoot without socks.

Walk

Participants will receive instructions to walk a distance of 6 meters across the force plate at their preferred speed, after familiarizing themselves with the markers. Each leg will undergo three trials, ensuring a successful strike on the force plate each time, which will then be averaged.

Balance

Two methods will be employed to assess static balance: Eyes Open Feet Apart (EOFA) and Eyes Open Feet Together (EOFT). Participants will be instructed to maintain stillness for three trials under each condition, each lasting 20 seconds.

The dynamic balance will be evaluated through three rounds each of the Functional Reach Test (FRT) and Lateral Reach Test (LRT) on both dominant and non-dominant sides. Average scores will be calculated from these trials for each test, and scores will be adjusted based on participant height for inter-individual comparisons.

Data management

The digital recruiting list will be securely stored and password-protected separately from participant identifiers. Access to this list will be restricted to the chief investigators. Registration records for MS will also be securely saved and password-protected. Personally identifiable information will only be used for confirming duplicate entries, after which names and phone numbers will be removed, leaving unique identification numbers. Interview participants will not be asked for personal information. Consent forms will be accessible only to the chief investigators, and stored securely in a locked cabinet. Personally identifiable information will never be shared, and results will be presented only in aggregate form. Study data will be accessible solely to the chief investigators.

Sample Size

Our power calculations are based on Borland et al. [63], who identified the MCID of SDMT for mild cognitive impairment. Borland et al. reported the MCID as -3.8 and the effect size as -0.3 for minimal change ≥0.5 in the Clinical Dementia Rating—Sum of Boxes for the SDMT. Based on Borland et al., and considering a significance level of 0.05 and 90% power, we will need a total of 190 participants using G*Power software. Assuming a dropout rate of around 20%, we will need 228 participants. Regardless of dropouts, we will conduct intention-to-treat analyses for all initially assigned patients. This involves considering all participants in the analysis, regardless of whether they complete the study.

Statistical analysis

Statistical analysis will be performed using SPSS version 23.0. Demographic characteristics, study characteristics, and baseline data will be summarized using descriptive statistics. The Statistical methods to be used for analyzing the study include two-way analysis of variance (ANOVA) to assess the main environmental effects (land and water), the effects of training conditions (DTT, combined exercise training, controlled), and any possible interaction effects between environmental and training conditions. Additionally, repeated measures ANOVA will be used to compare pre-post assessments within groups (land and water) and conditions (DTT, combined exercise training, control). The results will be reported according to the CONSORT Statement (2010) for the reporting of multi-arm factorial trials [64].

Ethics and dissemination

This study was approved by the Sport Sciences Research Institute (SSRI) (No. IR.SSRC.REC.1401.082) and adheres to the Declaration of Helsinki. Any protocol changes will be communicated to ethics boards and participating districts. Patients will sign informed consent forms. Results will be published in peer-reviewed journals and presented at MS conferences. Additional information will be available from the corresponding author post-publication.

Discussion

Although MS is not very common, its prevalence has significantly increased in recent years, especially since 2013 [9]. In Iran, the percentage change in age-standardized prevalence rates was reported as 40.4% between 1990 and 2016; however, during this period, the global percentage change in age-standardized prevalence rates was reported as 10.4% [65]. This underscores the importance of raising awareness and managing MS disease both nationally and globally. Exercise training is a valuable secondary strategy alongside pharmacological treatment. One notable exercise training method is "multidisciplinary rehabilitation" which combines exercise with non-exercise treatments, such as pairing medication with training exercises, performing exercises alongside cognitive activities (dual-tasking), or combining different types of exercise like endurance, strength, and balance exercises (combined exercise training) [16, 66].

Multidisciplinary rehabilitation acknowledges that a singular treatment is insufficient for addressing the varied symptoms of MS. It is likely that current interventions operate through separate pathways or mechanisms without overlap [16].

Combining exercise training with DTT provides a more precise representation of the complex demands of daily activities. These tasks replicate the challenges encountered during everyday tasks like driving, gardening, vacuuming, and walking pets, where individuals must perform cognitive and motor tasks simultaneously. For instance, while walking with a cup of water, participants engage both cognitive and motor functions, mirroring real-life scenarios that require focus and coordination. This kind of training enhances not only physical capabilities but also cognitive processing, crucial for maintaining independence in daily activities. Studies showed that both DTT and combined exercise training improved cognitive function, motor performance, walking capacity, walking speed, and dynamic balance [17, 22, 37, 38].

These interventions (combined and dual-tasks) can be performed in both water and land environments. Several studies have examined how aquatic interventions affect PwMS [24-27]. A meta-analysis found that aquatic training benefits both physical and mental well-being. This is particularly important for PwMS, who often experience depression and anxiety [31].

As far as we examined, no studies were studied the effects of cognitive and motor factors (kinetics and kinematics of walking and balance) on DTT and combined exercise training in aquatic and land environments. The dual-environmental approach of our study is particularly innovative; it allows for the assessment of how different settings impact the training outcomes and the functional applicability of the exercises. By exploring these environments, we aim to provide a comprehensive understanding of how specific training tasks can be utilized effectively in various real-life contexts.

As the prevalence of MS is increasing, heightened awareness may provide researchers and patients with a broader perspective on therapeutic exercises and effective symptom management in the future. In addition, these findings can help healthcare professionals prescribe and develop more effective exercise programs for PwMS. Furthermore, this study addresses important evidence gaps and provides clinical insights for MS management decision-making.

By emphasizing the ecological validity of our training tasks, we hope to demonstrate that these interventions are not merely theoretical but are grounded in practical, everyday applications that resonate with the challenges faced by PwMS.

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Figure Legends

Figure 1: **Trial profile.** Combined, Dual task, and Control group, with either in water or on land at baseline and endline.

Figure 2: **Intervention Training Protocol.** The 2*2*3 Factorial design, showing the 12-intervention training protocol based on combined, dual task, and control group with eighter in water or on land, baseline and endline.

Authors Contributions

S.N., S.S., and M.K. made substantial contributions to the conception and design of the study, including the development of the protocol and sample size calculations. S.N. and S.S. standardized the data collection forms and contributed to data collection and the initial draft and revision of this manuscript. M.K. will oversee data compilation, perform analysis, and contribute to data interpretation. All authors critically revised the manuscript for intellectual content, gave final approval of the version to be published, and agree to be accountable for all aspects of the work to ensure its accuracy and integrity. S.N. serves as the guarantor for this study.

The Alborz MS NGO will participate in the recruitment of patients based on the inclusion criteria. In order to evaluate adverse events and side effects, this association will participate. Also, cognitive pre and post-tests will be performed by neurologists of the Alborz MS Association.

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. further information will be accessible from the corresponding author after the completion of the trial.

Competing interest's statement

The study design, implementation, management, analysis, interpretation, and reporting of the study are entirely independent of the Kharazmi University and The Alborz MS NGO. The authors declare no conflicts of interest.

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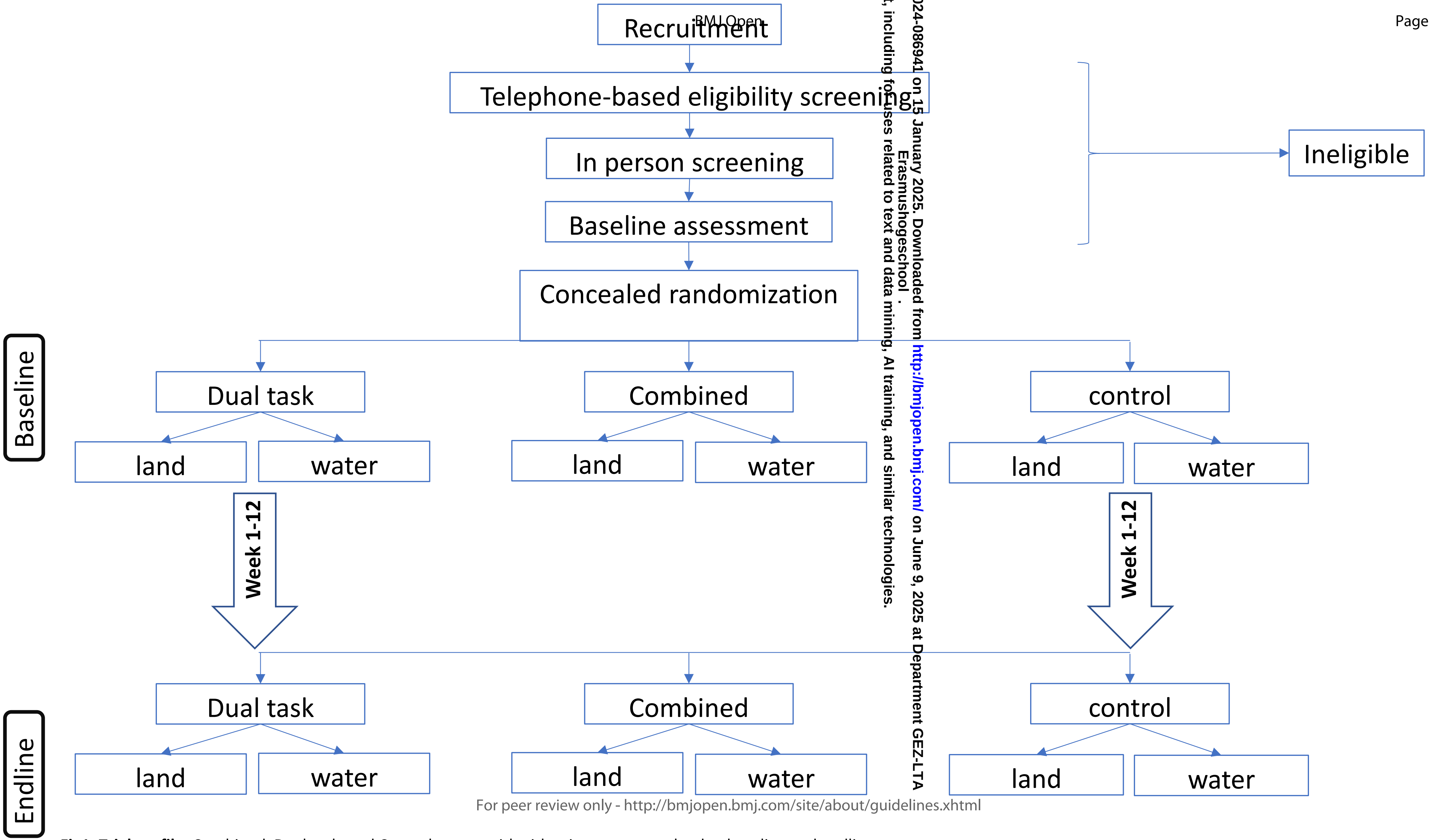

Fig1. Trial profile. Combined, Dual task, and Control group, with either in water or on land at baseline and endline

Intervention Type	environment	Before and after 12 weeks	
combined	In water	Baseline	Endline
		Baseline	Endline
	On land	Baseline	Endline
		Baseline	Endline
Dual task	In water	Baseline	Endline
		Baseline	Endline
	On land	Baseline	Endline
		Baseline	Endline
control	In water	Baseline	Endline
		Baseline	Endline
	On land	Baseline	Endline
		Baseline	Endline

Fig2.Intervention Training Protocol. The 2*2*3 Factorial design, showing the 12 intervention training protocol based on combined, dual task, and control group with eighter in water or on land, baseline and endline.

Comparative effects of dual-task training versus combined exercise training in water and on land on patients with multiple sclerosis: A Study protocol of a randomized factorial trial

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Comparative effects of dual-task training versus combined exercise training in water and on land on patients with multiple sclerosis: A Study protocol of a randomized factorial trial

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Abstract

Introduction: People with multiple sclerosis (PwMS) experience cognitive and motor impairments, including cognitive training to exercise training. This study compares dual-task and combined exercise training in water and on land. Water-based training may enhance cognitive and motor function more effectively than land-based training, presenting a promising intervention for PwMS. We explore dual-task training including cognitive and motor exercise with combined exercise in water and on land on the cognition, balance, and gait PwMS.

Methods and analysis: This is a double-blind 3*2*2 factorial randomized trial. Participants will be randomly allocated to one of six groups: four intervention groups and two control groups. All patients will receive supervised 12-week training sessions, twice a week, and will be assessed by a blinded outcome assessor before and at the end of the 12-week program. The primary outcome includes The Symbol Digit Modalities Test (SDMT). The secondary outcomes involve the California Verbal Learning Test-II (CVLT-II), the Brief Visuospatial Memory Test-Revised (BVMT-R), kinetics, and kinematics throughout the balance and gait cycle. All the data will be analyzed by a blinded data analyst.

Ethics and dissemination: Ethical approval was granted by the Sports Science Research Institute (SSRI) (No. IR.SSRC.REC.1401.082). The results of the trial will be submitted for publication in journals and distributed to PwMS and physiotherapists.

Trial registration number: The trial is prospectively registered on 2024/03/22, at https://www.umin.ac.jp/ with an identification number (UMIN000053947).

Keywords: Dual-task training, Combined exercise training; Multiple sclerosis; Cognition; Gait; Balance.

Strengths and limitations of this study

- Combined and dual-task exercise training is conducted in both water and land settings, allowing for a comparative analysis of environmental effects.
- Cognitive impairments and physical activity will be assessed using established cognitive assessment tools and comprehensive kinetics and kinematics measurements.
- While outcome assessors are blinded, participants and physical therapists will not be blinded to group allocation, which may introduce bias.
- The intervention follows a detailed, standardized protocol designed to ensure consistency across all training sessions.
- The study incorporates a robust system for monitoring adverse events, ensuring participant safety throughout the trial.

Introduction

Multiple sclerosis (MS) is a neurologic disease that adversely affects the central nervous system (CNS) through inflammation and demyelination. More than 2.8 million people worldwide are affected by MS, with a higher prevalence among women than men [1]. One of the most common phenotypes of MS is relapsing-remitting (RRMS) [2]. RRMS is typically diagnosed when PwMS have lesions in their nervous system or experience intermittent episodes of neurological symptoms involving inflammation over time [3], leading to demyelination, axon injury, loss of nerve cells, and subsequent atrophy [4]. Hence, PwMS often experiences a range of symptoms, including cognitive issues and motor dysfunctions. Studies investigated that 40-65% of PwMS and 30-45% of individuals with RRMS exhibit cognitive impairment [5, 6]. Common symptoms of cognitive impairment include decreased processing speed, deficits in learning and memory, perceptual skills, executive functions, and rarely linguistic or intellectual impairment [7]. Cognitive domains are crucial for executing motor tasks smoothly and managing complex actions. For instance, processing speed is vital for efficient signal transmission between the CNS and motor movement. Additionally, it predicts performance in executive functions and daily activities [8]. Executive functioning encompasses cognitive skills required for engaging in complex, goal-directed behavior and adapting to environmental demands, including abilities like planning and anticipating outcomes [8]. Therefore, deficits in cognitive domains can significantly impair motor function and the performance of daily activities in PwMS.

Due to the interaction between decreased physical and cognitive abilities, up to 75% of PwMS experience issues with balance and gait, both in the early stages and advanced stages of the disease [9]. Balance problems can also result from CNS damage or abnormal gait, leading to falls, injuries, loss of mobility, and reduced quality of life [10]. Moreover, there is evidence that balance depends not only on the integration of somatosensory, visual, and vestibular information but also on higher brain systems responsible for the memory needed for anticipatory movement [11].

Several studies have highlighted the walking difficulties in PwMS; Benedetti et al. reported that PwMS had a slower progression pace, shorter steps, and prolonged double support intervals during walking [12]. Coca-Tapia et al. showed a decrease in speed, step lengths, and stride lengths, alongside increased step width. They also noted decreases in hip extension during the stance phase, reduced knee flexion during the swing phase, decreased ankle dorsiflexion at initial contact, and decreased ankle plantar flexion during the preswing phase [13].

The decline in cognitive and physical performance significantly affects the daily activities and independence of PwMS [14], underscoring the critical role of symptom management. Managing symptoms is fundamental for promoting health and well-being in PwMS [15]. Exercise training is a valuable complement to disease-modifying drugs for managing MS and its symptoms [16], highlighting the need for effective non-pharmacological treatments like exercise therapy.

Unlike single-motor tasks, which may not fully represent daily activities, dual-tasks better simulate the cognitive and motor challenges of daily life [17]. Therefore, evaluating dual-task performance and tracking improvements can provide valuable insights into the overall functional status of PwMS. For instance, Kessler et al. found that difficulties in new learning significantly impacted activities of daily living, including shopping, housework, and transportation [18]. Additionally, safe functional movements necessitate simultaneous motor and cognitive tasks which are referred to as dual-task [19]. PwMS exhibit a reduced ability to perform dual-tasks [20], even in those with low EDSS scores [21]. It has been demonstrated that DTT has a moderately positive impact on enhancing dynamic balance and functional mobility in PwMS [22]. Additionally, it can improve gait speed, motor performance, and cognitive function in PwMS [17, 23].

Previous research indicates that aquatic training confers notable advantages over land-based training in MS research, notably due to buoyancy, viscosity, and thermodynamics [24]. PwMS commonly experience pain and reduced mobility [24]. Buoyancy mitigates joint impact, facilitating movements that may be challenging on land [25]. Also, Buoyancy mitigates muscle weakness and reduces the risk of fall-related injuries and fractures, prevalent among PwMS [26]. Studies have demonstrated that participating in aquatic activities can enhance muscle strength and reduce fatigue, both common concerns for PwMS [24, 27]. Viscosity provides drag forces, allowing multi-directional performance and offering low-impact resistance training, which can improve muscle strength and endurance [24, 27]. Land-based training also improves resistance; however, it can be adjusted by using weights or resistance bands.

Cerebral blood flow issues in PwMS lead to cognitive impairment, lesion formation, axonal degeneration, and fatigue [28]. Immersing in water may enhance cognitive function by promoting cerebral blood flow through hydrostatic pressure, stimulating mechanoreceptors, and increasing parasympathetic activity, which may positively influence attention and processing speed [29, 30]. Thermodynamics is advantageous due to the Uhthoff phenomenon, wherein MS symptoms exacerbate with increasing temperatures [31]. Water conducts heat 25 times faster than air, leading to more efficient regulation of body temperature during aquatic training compared to land-based exercises, due to water's superior heat retention capacity and faster heat transfer properties [24]. Previous studies have demonstrated the benefits of various cooling strategies before and after exercise training for PwMS [24, 32]. Among these strategies, engaging in aquatic exercise training in a cool pool stands out as an effective method to mitigate heat sensitivity.

Multiple studies have investigated the effectiveness of various exercise training interventions on in PwMS [26, 27, 33, 34]. However, a notable limitation is the tendency to focus on a singular mode of exercise training, neglecting the multifaceted physiological decline (e.g., aerobic deconditioning, muscle weakness, and balance dysfunction) in PwMS [35]. In addition to the previous emphasis on addressing balance deficiencies in PwMS [10], there is evidence suggesting that PwMS also experience decreases in both muscular and cardiorespiratory fitness levels. Lambert et al. found that PwMS exhibited significantly lower peak torque in various leg muscles compared to healthy individuals [33]. Similarly, Moatert et al. observed that cardiorespiratory fitness, measured by VO2peak, was 28% higher in healthy controls than in PwMS [34]. The decreasing fitness levels in PwMS, coupled with the vital importance of physical fitness for improving functionality and managing symptoms, highlight the critical need for concurrent interventions improving balance, gait, muscle strength, cardiovascular health, and cognitive functions [36-38]. Accordingly, Sandroff et al. [36] demonstrated that engaging in multimodal exercise training could enhance endurance walking performance and cognitive processing speed in PwMS experiencing significant mobility limitations. This improvement is likely attributed to enhancements in cardiorespiratory capacity. Motl et al. linked aerobic fitness with increased volumes of subcortical grey matter structures such as the hippocampus and basal ganglia [39], possibly explaining exercise's positive impact on ambulation and cognition [40]. We will therefore conduct a 12-week factorial trial to evaluate the effect of DTT and combined exercise training in water and land, on cognition, balance, and gait in PwMS.

Aims and hypothesis

The primary objective of this study is to compare the effects of DTT and combined exercise training in water and on land on cognitive impairment in PwMS and to examine the interaction effects of these treatments. Given that processing speed decline is central to cognitive impairment, it will be a key outcome to assess intervention effectiveness. Evaluating the effects of these interventions on gait kinetics and kinematics, including spatiotemporal parameters, sagittal plane angles, and moments of the hip, knee, and ankle, as well as anteroposterior, mediolateral, and vertical ground reaction forces in two different environmental conditions, remains secondary. The hypothesis posits that aquatic environments may enhance cognition and motor function more effectively than land environments. This is likely due to increased blood flow and mechanoreceptor stimulation during water immersion, which may help reduce cognitive impairment and enhance motor function. Another hypothesis suggests that combined exercise training may have a greater effect on gait kinetics compared to DTT. This is based on the importance of balance enhancement for improving gait, proprioception, coordination, and postural stability. Strengthening muscles is crucial for maintaining alignment and restoring the ability to respond quickly to stimuli, which can positively affect gait dynamics. Furthermore, aerobic exercises can enhance cardiovascular health, potentially increasing endurance during walking.

Method and Analysis

Study Design

This is a 2*2*3 factor randomized study with double-blind outcome assessor and the data analyst will be conducted at the campus of Kharazmi University, Tehran, Iran. The study is scheduled to begin on April 6, 2024, and is expected to conclude on July 29, 2024.

The trial was registered with the UMIN Clinical Trials Registry due to technical issues with the Iranian clinical trial registry at the time of registration. The decision to use UMIN was based on its availability and functionality. It is important to note that, although the registration occurred on a foreign platform, the trial is being conducted in Iran, following all national ethical and regulatory requirements, including ethical approval from the Sports Science Research Institute (SSRI) (No. IR.SSRC.REC.1401.082). Additionally, we had previous experience with UMIN from a prior study, which further influenced this choice.

The protocol is described according to Recommendations for Interventional Trials (SPIRIT) guidelines (Table 1), and the results of the study will be presented in a CONSORT statement (Fig. 1).

Table 1. SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) diagram of enrolment, interventions, and assessments of the trial.

	STUDY PERIOD					
	Enrolment	**Allocation**	**Post-allocation**			**Close-out**
TIMEPOINT	$-t_1$	**0**	**Week 4**	**Week 8**	**Week 12**	t_x
Enrolment						
Eligibility screen	X					
Informed consent	X					
Cognitive impairments assessment	X					
Safety for exercise clearance	X					
Allocation		X				
Interventions						
Dual-task in water			◆————	————◆		

Dual-task on land			⟷	⟷		
Combined in water			⟷	⟷		
Combined on land			⟷	⟷		
Control in water			⟷	⟷		
Control on land			⟷	⟷		
Clinical measures						
Weight, Height, BMI		X				
Level of education		X				
Symptoms duration		X				
Diagnosis duration		X				
Medication use		X				
Assessment						
BICAMS		X				X
Gait characteristics		X				X
Balance assessment		X				X
Safety (AEs)	X	X	X	X	X	X
Early withdrawal information	As required					
AEs: adverse events, BICAMS: Brief International Cognitive Assessment for MS						

Patient and public involvement

The study is crafted through extensive cooperation among PwMS, healthcare experts, and researchers. Additionally, representatives from the Alborz MS NGO of Iran, who are also PwMS, are involved as project partners to guarantee the benefits for patients.

Eligibility criteria

Participants are eligible to be included in the trial if they meet the following inclusion criteria: PwMS will be aged between 20 and 60, including both males and females, diagnosed with relapsing-remitting MS as outlined by the McDonald criteria [41], the EDSS scores ranging between 1.0 and 5.5 [42], Relapse-free for the last 3 months [43], have not engaged in regular physical exercise (no training within the last three months), be willing to participate in the current study, Cognitive impairments that will be characterized by scores below the standard criterion of at least 1.5 standard deviations [8] on at least one assessments from the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) [44].

Potential participants will be excluded if they are unable to comply with the requirements of the protocol, inability to stand and walk without aids and braces, changes in medication in the past 2 months [45], history of cardiovascular, kidney, or other chronic diseases, pregnancy in the past 12 months [45], medical conditions interfering with mobility, MS-like syndromes such as neuromyelitis optica, or major problems with hearing, vision, and perception [46].

Recruitment

We will recruit PwMS from the Alborz MS NGO of Iran. Community-based recruitment will be conducted through a multi-channel approach, involving collaboration with PwMS themselves, referrals from neurologists, and advertisements via both traditional (e.g., hospital boards, printed flyers) and digital platforms (e.g., Instagram, social media groups). Based on the inclusion criteria, the researchers will enroll eligible PwMS in the study. At enrollment, contributors will be randomly assigned to one of six groups: dual-task training in water, dual-task training on land, combined exercise training in water, combined exercise training on land, control group in water, and control group on land (Fig. 2).

Screening

Volunteers will undergo a telephone assessment to determine eligibility. Those meeting the criteria will be invited for a screening appointment at the trial center. This includes MS EDSS evaluation, medication history questionnaires, exercise safety clearance, assessment of baseline physical activity, and evaluation for cognitive impairment by a neurologist.

Safety for Exercise Clearance

A pre-exercise assessment will identify PwMS at higher risk of adverse events during exercise. Functional assessments will be utilized to develop exercise program recommendations, aligned with American Sports Medicine Association guidelines [47]. These include the six-minute walk test (6MWT), where patients walk as fast as they comfortably can for 6 minutes without running or jogging, aimed at measuring functional endurance [48]. The timed 5-repetition sit-to-stand assesses the time taken to complete five repetitions of the sit-to-stand movement as quickly as possible, serving as a measure of strength [49]. Furthermore, the Berg Balance Scale evaluates patients' performance on 14 items, comprising 5 static and 9 dynamic items, related to balance function frequently encountered in everyday life [50].

Safety

We will monitor adverse events (AEs) during the trial, which are side effects lasting over two days or requiring extra treatment. Examples include injury, illness, falls, joint pain, upper respiratory tract infections, sprains, strains, and muscle pain. Adherence reminder sessions will occur face-to-face, covering potential adverse events and appropriate actions, emphasizing contacting the clinic for relevant symptoms. Follow-up sessions will address participant concerns and reinforce key messages from the initial session. Significant AEs will be reported to chief investigators within one day. Participants experiencing AEs will cease intervention and be withdrawn from the study.

Randomization

An independent researcher will use computer-generated random sequencing to allocate participants in a 1:1:1:1:1:1 ratio via http://randomizer.org. Randomization will follow a blocked model with a block size of 24, concealed in numbered, sealed envelopes. Group allocation will be revealed to clinicians just before the first session. Participants will not be blinded due to the nature of the intervention.

Intervention

Licensed physical therapists experienced in managing PwMS will conduct interventions. Each group will have supervised sessions twice a week for 12 weeks, totaling 24 sessions. Each session will be approximately 60 minutes long, with regular breaks. All interventions, including dual-task training, combined exercise training, and the control intervention, will be applied in both environments: land and water.

Water Environment

The pool depth is 120 cm, approximately aligned with the participants' xiphoid process level. Water temperature will be maintained at a constant 30–31°C, while the room temperature will be kept at 26–28°C [51].

Land environment

Electric coolers and cold neck packs will be provided to reduce the risk of hyperthermia and symptomatic fatigue during exercise.

Dual-task training

DTT will integrate motor tasks with cognitive tasks. In this study, DTT is founded on Veldkamp et al. with a few modifications [52]. Motor activities will encompass walking at a preferred speed, fast walking, change in gait speed, backward walking, walking with a full cup of water, tandem walking, plantarflexion walking, and dorsiflexion walking, concurrently with cognitive tasks. Motor tasks are chosen based on prior research investigating reliability among individuals with neurological disorders engaging in various walking activities [43, 53, 54]. Cognitive functions addressed include fluency, working memory, selective attention, auditory discrimination, processing speed, executive function, text comprehension, sustained attention, auditory memory, visual discrimination, visual-spatial imagery tasks, and verbal and visual reasoning. All domains are selected due to their demonstrated early impairment in the PwMS [7, 8]. During the session, participants will be allowed adequate rest between exercises to manage their fatigue. The DTT protocol is detailed in Table 2.

For the baseline, participants' steps will be tracked while performing various walking tasks using a pedometer. Following each DTT, their steps will be tracked again, and they will receive feedback on their performance based on answer accuracy and steps tracked. Participants will advance to a higher difficulty level if they maintain ≥70% of baseline steps per minute of DTT and achieve ≥70% accuracy in their responses. If the number of steps per minute of DTT falls below <50% of the baseline, participants will be instructed to return to a lower difficulty level. This instruction is necessary if the accuracy of responses also falls below <50%, or if it is deemed necessary by the

practitioner for safety or quality reasons [55].

Table 2. Dual-task training

Motor function	**Exercise description / Difficulty levels**
Preferred walking speed	Walking with preferred speed for 2 min.
Fast walking speed	1. for 30 seconds. 2. for 60 seconds. 3. for 90 seconds.
Change in walking speed	1. Perform for 2 minutes with a 30-second interval, and an execution time of 10 seconds. 2. Perform for 2 minutes with a 20-second interval, and an execution time of 10 seconds. 3. Perform for 3 minutes with a 30-second interval, and an execution time of 10 seconds.
Backward walking	Walking with preferred speed for 1 min.
Walking with full cup of water	1. Walking with preferred speed for 1 minutes. 2. Walking with fast speed for 30 seconds.
Tandem walking	2. for 2 minutes. 3. for 1 minutes.
Plantarflexion walking	Walking for 30 seconds.
Dorsiflexion walking	Walking for 30 seconds.
Cognitive function	**Exercise description / Difficulty levels**
Working memory, Verbal fluency	Say a word to follow guidelines. 1. the word's first letter is given 2. the word's last letter is given. 3. the word's 2nd letter is given.
Working memory, Selective attention, Auditory discrimination	recognize words that are: 1. semantically different among other words. 2. from the same category. 3. from the same category or semantically different.
Working memory Executive function	1. Spell a 4- letter word backward. 2. Spell a 4 to 6, letter word backward. 3. Spell a 7+ letter word in backward.
Working memory	reverse the order of stating numbers, days, or months (e.g. Wednesday, Tuesday, Monday, etc.).
sustained attention processing speed	subtract 3 repeatedly from a hundred. subtract 4 repeatedly from a hundred. subtract 7 repeatedly from a hundred.

working memory **processing speed** **verbal analog reasoning**	Combine language and computation "If Wednesday is the 15th, what will the date of next Saturday be?" "What day will it be in three days if it is the 15th of the month today?"
Verbal fluency **Executive function**	Explain a word, without 1. directly mentioning it. 2. utilizing derived vocabulary. 3. resorting to gestures. 4. refraining from using abbreviations or hints implying its sound.
Text comprehension **Verbal memory** **Sustained attention**	answer the questions after listening to the story: 1. 3 multiple-choice (3 options) questions about the story. 2. 3 multiple-choice (4 options) questions about the story.
Auditory memory (recognition)	Two series of words are read. Figure out if any word heard in the second sequence has been heard before or not.
executive function **Visual imaginary spatial task**	name the opposite direction of the actions. say the word "left" when they move their right hand.
Selective attention	Stroop task name the color of a printed word while ignoring the word's actual meaning e.g., the word "RED" written in blue ink is blue.
Auditory discrimination **Working memory** **Selective attention**	recognizing the sounds or voices from a compact disc 1. voices (man, woman, child). 2. sounds (bicycle bell, car start, dog bark, …).
Visual discrimination	Similarities and differences between the two images. 1. more than one difference (simple images). 2. one difference (difficult images). 3. more than one difference (difficult images).
Visual imaginary spatial task	Imagine the direction of the path (the route from their residence to the swimming pool or gym)
Verbal and visual analog reasoning	calculation assignment adding or subtracting from letters (e.g., M + 2= O).

Combined Exercise training

Prior to main training, there will be an initial warm-up that includes exercises targeting the lower and upper limbs (squats, lateral lunges, horizontal arm extension, and vertical arm extension using resistance bands). The protocol will follow the recommendations of Kim et al. (2019) and Latimer-Cheung et al. (2017) [56, 57], in which sets, repetitions, and loads (averaged based on the percentage of the 1 repetition maximum) are defined as light (up to 40%), moderate (between 40% and 60%), and high (up to 60–80%).

The macrocycle training will progress as follows: the first and second weeks will consist of 2 sets of 10 reps with a light load; this will transition to 2 sets of 10 reps with a light to moderate load in the third and fourth weeks; The fifth and sixth weeks will consist of 2 sets of 12 reps with a moderate load; Weeks seven and eight will consist of 3 sets of 10 repetitions with a moderate load; Weeks nine and ten will include 2 sets of 10 reps with a moderate to high load;

and finally, weeks eleven and twelve will consist of 2 sets of 8 reps with a high load. The rest period between sets and muscle groups will be approximately 2 minutes.

Aerobic training involves 10 minutes of cycling on an ergometer, maintaining a heart rate (HRmax) of 40-70% according to the Karvonen formula: exercise HR = percentage of target intensity (HRmax - HRrest) + HRrest. Patients will utilize a heart rate monitor (Polar H9, Finland) during each session. Progression in intensity will be determined based on the tolerance of the PwMS. Patients will be encouraged to communicate their sensations during the exercise sessions, while the assistant will also observe their facial expressions for signs of discomfort to ensure the sessions' duration and frequency are well tolerated.

Balanced training will be conducted based on three balanced dimensions:

1. Stable base of support (BOS): Maintaining a stable base of support throughout the balance training by utilizing the center of mass. 2. Sway: Voluntarily shifting the center of mass to the limits of stability. 3. Step and walk: Creating and managing a new base of support by intentionally shifting the center of gravity beyond the stability limit.

Balance training will comprise 10-minute exercises, each lasting 15 seconds. These exercise training progress from easy to challenging, based on Sattelmeyer et al. (2021) [58]. The protocol concludes with stretching and cooling down major muscle groups. For detailed protocol information, refer to Table 3.

Table 3. Combined exercise training

	Type	Frequency	Intensity	Time
Warm-up	low-intensity walking	2 days per week	Up to 30% HRpeak	5-10 mi
Strength training	Squats, Lateral Lunges, Horizontal arm extension, Vertical arm extension using resistance bands.	2 days per week	light (up to 40%) medium (between 40% and 60%) high (up to 60–80%) 2-3 sets between 8–12 repetitions of each .	30-25 mi
Aerobic training	Cycling on an ergometer	2 days per week	40–70% HRpeak	10 min

Balance training	Standing wide stance Standing feet together Step stance wide feet position Semi-tandem stance Stable Tandem stance feet apart Stable Tandem stance Stable One leg stance Stable Wall leaning forwards Wall leaning backwards Standing moving body sidewards Rolling ball forwards Stepping forwards Stepping sidewards Stepping backwards Leaning forward reactive step Line walking Walk backwards Heel walking Forefoot walking	2 days per week	2-3 balance exercises each session, Progressing from dimension 1 to dimension 3.	10 min each exercise
Stretch and cool down	Static stretching of major muscle groups	2 days per week	Stretch to the point of feeling tightness	5 min Hold 30-6 2-3 repetit

Control interventions

This will include 60-minute maximum supervised cycling sessions on an ergometer. Participants are free to take a break whenever they desire, with an intensity set at 30–40% of their maximum heart rate. A great deal of PwMS is advised to utilize a cycle ergometer as it requires less balance and coordination than walking on a treadmill [59]. Moreover, continuous walking for an hour increases the possibility of fatigue, monotony, and adverse events for PwMS. Therefore, selecting the cycle ergometer as a comparator is justified.

Assessment

Each participant will undergo evaluation by an independent, trained, blinded assessor who will be unaware of the group allocation. There will be two separate assessments utilizing approved and validated outcome measures: one at baseline and one after the intervention.

Demographic and diagnostic baseline data

All participants will have their age, gender, weight, height, BMI, level of education, number of years since the onset of symptoms, duration since diagnosis, the six-minute walk test (6MWT), the timed 5-repetition sit-to-stand, and Berg Balance Scale recorded on a standardized data collection form. In addition, data on the medications currently being taken and those taken over the research period will be acquired.

Outcomes

The Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) is a measurement tool that will be used to assess cognitive impairment. It will be administered in one session by a neurologist in a quiet room and typically takes about 15 minutes to complete. The BICAMS includes three tests: the Symbol Digit Modalities Test (SDMT), the California Verbal Learning Test-II (CVLT-II), and the Brief Visuospatial Memory Test-Revised (BVMT-R) [60, 61].

Primary outcomes

The Symbol Digit Modalities Test (SDMT)

The SDMT measures processing speed, which is the primary cognitive impairment in PwMS. Participants will receive a series of nine meaningless geometric symbols, each numbered one through nine and will be required to orally match these symbols with the corresponding numbers in the correct sequence within a ninety-second timeframe. The score equals the number of correct substitutions.

Secondary outcome

California Verbal Learning Test-II (CVLT-II)

The test will involve participants listening to a list of 16 words read by the examiner. They will then attempt to recall as many words as possible in any order. After the initial recall, the list will be read again, and participants will attempt to recall more words. This process will be repeated for five trials.

Brief Visuospatial Memory Test-Revised (BVMT-R)

Participants will view six abstract designs for 10 seconds each, then mark interpretations on paper. Each design earns 0 to 2 points based on accuracy and location, totaling 0 to 12 points. The test includes three trials, measuring performance by total points earned.

Nationality influence on BICAMS

Smerbeck et al. [62] examined the influence of nationality on BICAMS in PwMS across multiple countries including Iran. They found significant impacts on all three tests due to variations in test formats and socio-cultural contexts, including language effects and quality of translations. Factors such as educational backgrounds and linguistic nuances, such as familiarity with left-to-right languages, could influence performance. Therefore, when implementing BICAMS globally, including norm-setting, accounting for nationality is essential [62].

Kinematics and kinetics

Kinematic and kinetic data will be collected at 120 Hz using 6 Vicon MX T40-S cameras, Vicon Workstation software (Oxford Metrics Ltd., Oxford, UK), and 2 Kistler force plates (Kistler Instruments AG, Winterthur, Switzerland). Individual spherical 14 mm retro-reflective markers will be placed as follows: C7, T10, clavicle : On the jugular notch where the clavicles meet the sternum, Sternum: On the xiphoid process of the sternum, shoulder: On the acromio-clavicular joint, finger: Just proximal to the middle knuckle on the hand, anterior superior iliac spine (ASIS), posterior superior iliac spine (PSIS), left thigh: Over the lower lateral 1/3 surface of the thigh, right thigh: Over the upper lateral 1/3 surface of the right thigh, knee: On the Lateral Epicondyle of the knee, left tibia: Over the lower 1/3 surface of the shank, right tibia: Over the upper 1/3 surface of the right shank, ankle: On the lateral malleolus along an imaginary line that passes through the transmalleolar axis, heel: On the calcaneus at the same height above the plantar surface of the foot as the toe marker, toe: Over the second metatarsal head, on the mid-foot side of the equinus break between the forefoot and mid-foot, for both the left and right sides.

The Vicon Workstation software will be utilized for data processing, encompassing joint kinematics and kinetics calculations, with code generated by Vicon Bodybuilder. Kinetic data will be normalized relative to body mass. Essential spatiotemporal parameters (stride time, cadence, gait speed, and stride length), alongside measures of relative phase (stance phase, swing phase, double stance phase, single support), will be extracted from all systems. Sagittal plane angles and moments at the hip, knee, and ankle throughout the gait cycle will be evaluated. Additionally, anteroposterior, mediolateral, and vertical ground reaction forces will be assessed. All testing sessions will entail patients being barefoot without socks.

Walk

Participants will receive instructions to walk a distance of 6 meters across the force plate at their preferred speed, after familiarizing themselves with the markers. Each leg will undergo three trials, ensuring a successful strike on the force plate each time, which will then be averaged.

Balance

Two methods will be employed to assess static balance: Eyes Open Feet Apart (EOFA) and Eyes Open Feet Together (EOFT). Participants will be instructed to maintain stillness for three trials under each condition, each lasting 20 seconds.

The dynamic balance will be evaluated through three rounds each of the Functional Reach Test (FRT) and Lateral Reach Test (LRT) on both dominant and non-dominant sides. Average scores will be calculated from these trials for each test, and scores will be adjusted based on participant height for inter-individual comparisons.

Data management

The digital recruiting list will be securely stored and password-protected separately from participant identifiers. Access to this list will be restricted to the chief investigators. Registration records for MS will also be securely saved and password-protected. Personally identifiable information will only be used for confirming duplicate entries, after which names and phone numbers will be removed, leaving unique identification numbers. Interview participants will not be asked for personal information. Consent forms will be accessible only to the chief investigators, and stored securely in a locked cabinet. Personally identifiable information will never be shared, and results will be presented only in aggregate form. Study data will be accessible solely to the chief investigators.

Sample Size

Our power calculations are based on Borland et al. [63], who identified the MCID of SDMT for mild cognitive impairment. Borland et al. reported the MCID as -3.8 and the effect size as -0.3 for minimal change ≥0.5 in the Clinical Dementia Rating—Sum of Boxes for the SDMT. Based on Borland et al., and considering a significance level of 0.05 and 90% power, we will need a total of 190 participants using G*Power software. Assuming a dropout rate of around 20%, we will need 228 participants. Regardless of dropouts, we will conduct intention-to-treat analyses for all initially assigned patients. This involves considering all participants in the analysis, regardless of whether they complete the study.

Statistical analysis

Statistical analysis will be performed using SPSS version 23.0. Demographic characteristics, study characteristics, and baseline data will be summarized using descriptive statistics. The Statistical methods to be used for analyzing the study include two-way analysis of variance (ANOVA) to assess the main environmental effects (land and water), the effects of training conditions (DTT, combined exercise training, controlled), and any possible interaction effects between environmental and training conditions. Additionally, repeated measures ANOVA will be used to compare pre-post assessments within groups (land and water) and conditions (DTT, combined exercise training, control). The results will be reported according to the CONSORT Statement (2010) for the reporting of multi-arm factorial trials [64].

Ethics and dissemination

This study was approved by the Sport Sciences Research Institute (SSRI) (No. IR.SSRC.REC.1401.082) and adheres to the Declaration of Helsinki. Any protocol changes will be communicated to ethics boards and participating districts. Patients will sign informed consent forms. Results will be published in peer-reviewed journals and presented at MS conferences. Additional information will be available from the corresponding author post-publication.

Discussion

Although MS is not very common, its prevalence has significantly increased in recent years, especially since 2013 [9]. In Iran, the percentage change in age-standardized prevalence rates was reported as 40.4% between 1990 and 2016; however, during this period, the global percentage change in age-standardized prevalence rates was reported as 10.4% [65]. This underscores the importance of raising awareness and managing MS disease both nationally and globally. Exercise training is a valuable secondary strategy alongside pharmacological treatment. One notable exercise training method is "multidisciplinary rehabilitation" which combines exercise with non-exercise treatments, such as pairing medication with training exercises, performing exercises alongside cognitive activities (dual-tasking), or combining different types of exercise like endurance, strength, and balance exercises (combined exercise training) [16, 66].

Multidisciplinary rehabilitation acknowledges that a singular treatment is insufficient for addressing the varied symptoms of MS. It is likely that current interventions operate through separate pathways or mechanisms without overlap [16].

Combining exercise training with DTT provides a more precise representation of the complex demands of daily activities. These tasks replicate the challenges encountered during everyday tasks like driving, gardening, vacuuming, and walking pets, where individuals must perform cognitive and motor tasks simultaneously. For instance, while walking with a cup of water, participants engage both cognitive and motor functions, mirroring real-life scenarios that require focus and coordination. This kind of training enhances not only physical capabilities but also cognitive processing, crucial for maintaining independence in daily activities. Studies showed that both DTT and combined exercise training improved cognitive function, motor performance, walking capacity, walking speed, and dynamic balance [17, 22, 37, 38].

These interventions (combined and dual-tasks) can be performed in both water and land environments. Several studies have examined how aquatic interventions affect PwMS [24-27]. A meta-analysis found that aquatic training benefits both physical and mental well-being. This is particularly important for PwMS, who often experience depression and anxiety [31].

As far as we examined, no studies were studied the effects of cognitive and motor factors (kinetics and kinematics of walking and balance) on DTT and combined exercise training in aquatic and land environments. The dual-environmental approach of our study is particularly innovative; it allows for the assessment of how different settings impact the training outcomes and the functional applicability of the exercises. By exploring these environments, we aim to provide a comprehensive understanding of how specific training tasks can be utilized effectively in various real-life contexts.

As the prevalence of MS is increasing, heightened awareness may provide researchers and patients with a broader perspective on therapeutic exercises and effective symptom management in the future. In addition, these findings can help healthcare professionals prescribe and develop more effective exercise programs for PwMS. Furthermore, this study addresses important evidence gaps and provides clinical insights for MS management decision-making.

By emphasizing the ecological validity of our training tasks, we hope to demonstrate that these interventions are not merely theoretical but are grounded in practical, everyday applications that resonate with the challenges faced by PwMS.

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Figure Legends

Figure 1: **Trial profile.** Combined, Dual task, and Control group, with either in water or on land at baseline and endline.

Figure 2: **Intervention Training Protocol.** The 2*2*3 Factorial design, showing the 12-intervention training protocol based on combined, dual task, and control group with eighter in water or on land, baseline and endline.

Authors Contributions

S.N., S.S., and M.K. made substantial contributions to the conception and design of the study, including the development of the protocol and sample size calculations. S.N. and S.S. standardized the data collection forms and contributed to data collection and the initial draft and revision of this manuscript. M.K. will oversee data compilation, perform analysis, and contribute to data interpretation. All authors critically revised the manuscript for intellectual content, gave final approval of the version to be published, and agree to be accountable for all aspects of the work to ensure its accuracy and integrity. S.N. serves as the guarantor for this study.

The Alborz MS NGO will participate in the recruitment of patients based on the inclusion criteria. In order to evaluate adverse events and side effects, this association will participate. Also, cognitive pre and post-tests will be performed by neurologists of the Alborz MS Association.

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. further information will be accessible from the corresponding author after the completion of the trial.

Competing interest's statement

The study design, implementation, management, analysis, interpretation, and reporting of the study are entirely independent of the Kharazmi University and The Alborz MS NGO. The authors declare no conflicts of interest.

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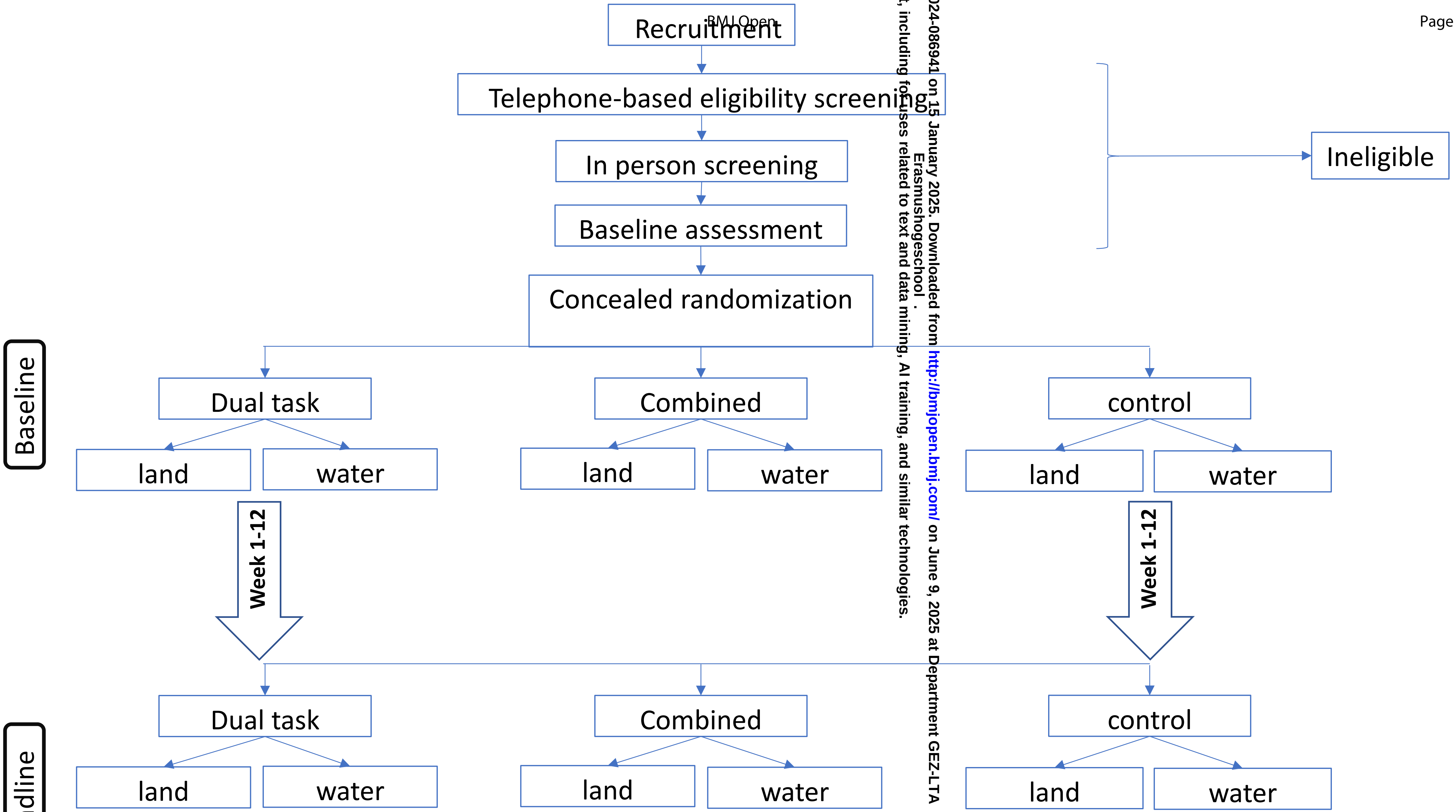

Fig1. Trial profile. Combined, Dual task, and Control group, with either in water or on land at baseline and endline

Intervention Type	environment	Before and after 12 weeks	
combined	In water	Baseline	Endline
		Baseline	Endline
	On land	Baseline	Endline
		Baseline	Endline
Dual task	In water	Baseline	Endline
		Baseline	Endline
	On land	Baseline	Endline
		Baseline	Endline
control	In water	Baseline	Endline
		Baseline	Endline
	On land	Baseline	Endline
		Baseline	Endline

Fig2.Intervention Training Protocol. The 2*2*3 Factorial design, showing the 12 intervention training protocol based on combined, dual task, and control group with eighter in water or on land, baseline and endline.