**ORIGINAL ARTICLE** 



# The effectiveness of cognitive-motor training on reconstructing cognitive health components in older male adults, recovered from the COVID-19

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## Abstract

**Objective** The incidence of COVID-19 disease in the elderly can accelerate normal degenerative process of cognitive functions. Interactive cognitive-motor training (CMT) is an intervention that integrates cognitive and motor tasks to promote individuals' physical and psychological health. The present study aimed to examine the effect of CMT on reconstructing cognitive health components in older men, who have recently recovered from COVID-19.

**Materials and methods** This study is a quasi-experimental repeated measure (without control group). Participants were 42 elderly men (65–80 years) who recovered from the COVID-19 disease that individually participated in a 4-week CMT program twice a week. The cognitive health components of the participants were assessed by the General Health Questionnaire (GHQ-2) and the Mini-Mental State Examination (MMSE) at 3 stages before the beginning of the intervention (baseline assessment); 2 weeks after the intervention (short-term follow-up); and 3 months after the intervention (long-term follow-up). **Results** The results showed that the scores of depression, anxiety, physical symptoms, and social performance components and the overall GHQ score improved significantly in short-term follow-up (P < 0.05) and also in long-term follow-up compared to baseline assessment (P < 0.05). It was also found that attention and calculation, recall, lingual skill, and action performance components and the overall score of MMSE were also improved at three stages of assessments. Other components did not differ among stages.

**Conclusions** This study adds to the research on the effectiveness of using CMT for reconstructing cognitive health components in older adults, recovered from the COVID-19, and supports CMT as a viable intervention practice.

Keywords General health · Cognitive function · Dual-task · Training · COVID-19 · Elderly

# Introduction

People around the world are exposed to the COVID-19 virus and the complications caused by it. However, older people are more likely to be affected by the virus than other age

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<sup>2</sup> Exercise Physiology Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran groups [1]. Older adults are also more likely to experience various symptoms caused by the COVID-19 virus. In fact, older people typically experience problems such as seriousness of disease, lack of access to regular outpatient visits, lack of medication management, social isolation, cognitive decline, and decline in mental health [2].

After remission from COVID-19 symptoms, cognitive, mental, and physical disorders can make the elderly very vulnerable [3]. This has a significant impact on their cognitive and mental health. Decreased function of various organs of the elderly after fighting the COVID-19 causes many changes in factors related to the health of the elderly and provides the conditions for their subsequent complications [4].

During the current COVID-19 crisis, many countries have begun isolating, quarantining, and staying at home [5]. Although this procedure reduces the prevalence of the disease, it can cause further problems for the elderly, especially in those who have recently survived COVID-19 and now are exposed to mental, physical, and cognitive weakening. Therefore, planning to prevent the occurrence of such outcomes after the disease is one of the measures that can help the elderly [1].

In addition to traditional medical treatment, interventions that develop multiple aspects of cognitive and motor readiness may be beneficiary [6]. Training that used dual-tasking paradigms demonstrated beneficial effects on cognitive and motor control in older adults [7, 8], in patients with brain injury [9, 10], and in patients with Alzheimer's disease [11]. Several studies have suggested that procedures to improve the dual-task performance of the elderly should be included in physical and psychological disorder prevention programs [12–15].

Kitazawa and colleagues (2015) used a dual-task netstep exercise (NSE) to improve cognitive functions in older adults and showed that dual-task NSE is capable of improving cognitive performance in healthy older adults [12]. Bisson and colleagues (2007) also examined the effect of virtual reality (VR) and biofeedback (BF) training on balance and reaction time in older people. They found that postural sway during quiet stance did not change significantly; however, significant improvements on the community balance and mobility scale (CB & M) as well as decreased reaction times with VR and BF training were observed [13]. Similarly, Nishiguchi and colleagues (2015) in the investigation of healthy older adults discovered that a physical and cognitive program can improve cognitive function and brain activation efficiency [14]. Also, Schoene and colleagues (2013) showed the effectiveness of a step-based exercise game on cognitive functions associated with falls [16]. In this regard, Morita and colleagues (2018) showed the effect of 2-year cognitive-motor dual-task (DT) training on cognitive functions and motor ability in healthy elderly people [15]. There is evidence to suggest that combined cognitive and motor trainings may lead to cognitive enhancement and improve elderly's independence. On the other hand, these exercises can lead to increased self-confidence, relaxation, and functional facilitation in the individual [17, 18].

As people get older, their ability to do multiple tasks at the same time decreases [19]. COVID-19 disease also reduces the ability of the elderly to focus their attention, resulting in more difficulty in performing a dual task. To perform different tasks, one needs to divide one's attention, which may interfere with the control of motor and cognitive behaviors [20].

Accordingly, it is expected that the cognitive-motor training method has an appropriate and stable effect on the reconstruction of cognitive functions in the elderly who recovered from the COVID-19 disease. Therefore, the purpose of the present study was to investigate the effect of cognitive-motor training on the components of cognitive health of the elderly who recovered from COVID-19.

## **Materials and methods**

This study is a quasi-experimental, with a repeated measures design and without a control group.

#### **Participants and eligibility**

A number of 42 male participants aged 65-80 years old, living in the community and recently discharged from a hospital in Tehran, volunteered to take part in this study with the consent from their physician. The eligibility criteria included having 65 years of age or older, being able to read and write, living in the Tehran metropolitan area, being independent in activities of daily life, being able to walk 10 m without using a walking aid, and willingness to provide informed consent and to comply with the study protocol. Also, the severity of COVID-19 disease was set at stage 1, with symptoms including headache, loss of sense of smell, cough, fever, hoarseness, chest pain, and fatigue. Those with more severe symptoms were not admitted into the study. Exclusion criteria included an acute psychiatric condition with psychosis, an unstable medical condition that would preclude safe participation, a progressive neurological condition (such as Parkinson's disease, multiple sclerosis, Meniere's disease), cognitive impairment defined as a Pfeiffer Short Portable Mental Status Questionnaire (SPMSQ) score < 824, or visual or auditory impairment that could not be corrected with assistive devices.

Potential participants undertook an initial eligibility screening via a telephone interview. This included oral screening using SPMSQ. Trained research personnel provided detailed study information and obtained verbal consent to arrange an appointment for a baseline assessment. Study information also was posted to potential participants at this time.

Immediately before a scheduled baseline assessment, participants were asked to watch a video showing the main aspects of the intervention to establish their intention to adhere to the training protocol. As participants showed their unwillingness to adhere with the intervention protocol, they were excluded from the study. Written consents were obtained from those who were willing to participate in the study. Following the baseline assessment (GHQ-2 and MMSE), each participant was subjected to a single-subject research design. According to the social distancing protocol, the intervention for each participant was performed individually at his residence.

#### Sample size

A sample size calculation (5% significance level, 80% power, 33% effect, 20% dropout rate) was performed using the nbpower command in STATA version 16 which indicated that a sample of 36 is necessary to achieve 80% power. Therefore, this number of participants was chosen from primary 86 volunteers (Fig. 1).

## Interventions

The training period lasted 4 weeks, and each week consisted of two exercise sessions.

The training protocol included physical exercises with low to high cognitive load and had two types of challenging requirements: (1) motor requirement such as shifting the center of gravity, consecutive walking, and moving the limb in full range of motion and (2) cognitive requirement such as attention, quick response to visual stimuli, decision-making, and response inhibition (Fig. 2). The intensity and duration of the program were selected according to the guidelines of the American College of Sports Medicine and previous studies [21], which showed that 1- to 5-h dual-task training programs (motor training and cognitive training) were effective in improving motor function and psychological performance in older adults, respectively [22–24].

In order to comply with the "social distancing protocols," training sessions were conducted individually and at the participants' residence to prevent the possibility of re-emergence of the disease. Each training session lasted an average of 45 min and included 6 exercises in 2 to 3 sets (5–10 repetitions per set). Participants underwent a 10-min training session at each station before rotating on until all exercises were completed. All participants received the same amount of contact time with each trainer. A family member of the participants was also asked to, after complete training, supervise the whole procedure. As the present research adopted a quasi-experimental design, we tried as much as possible to control possible confounders (e.g., natural change of participant's state over time, change in the infection status); fortunately, no case was reported.

The provision of educational materials involved a followup telephone call to monitor participants' involvement in the exercises. The intensity of the exercise was controlled using the amount of perceived pressure by the participants. The number of repetitions and the cognitive load of the exercise increased as the participants progressed. Therefore, the training program was designed to include three levels (A, B, and C), in which the motor and cognitive load gradually increased from level A (minimum load) to level C (maximum load).

All participants started the exercises in level A and only entered the next level after complete success in this level. Motor training protocol included standing on the support surface, walking around obstacles, hitting the ball while standing, throwing the ball into the basket while standing,



**Fig. 1** Profiles of the stages of this study



**Fig. 2** The effects of cognitive-motor training on general health ((a) depression, (b) anxiety, (c) physical symptoms, (d) social performance, and (e) the overall scores) at various stages of the test. \*Significance level  $P \le 0.05$ 

walking and hitting the ball, walking in a zigzag path while holding a ping pong ball, and walking on a narrow support surface while holding an object. The cognitive training included countdown, reverse spelling, and poem reading [21], and these were done verbally.

## **Evaluations**

Assessments included General Health Questionnaire (GHQ-2) and Mini-Mental State Examination (MMSE). The General Health Questionnaire (GHQ-2) is a self-administered screening questionnaire that has been designed to measure the psychological aspect of health [25]. The Mini-Mental State Examination (MMSE) is a widely used test of cognitive functions among the elderly; it includes tests of orientation, attention, memory, language, and visual-spatial skills [26].

#### Statistical analysis

The results of the Shapiro–Wilk test indicated the normal distribution (P = 34) and the result of the Levene test indicated the homogeneity of variance of the data in different stages of the test, respectively [GHQ-2: F = 1.91 and P = 0.17 > 0.05] and [MMSE: F = 0.21 and P = 0.26 > 0.05]. Box's test was used to determine whether variance–covariance matrices are equal [Initial evaluation: Box's M = 15.41, F = 2.18, P = 0.06 > 0.05], [Two weeks after the intervention: Box's M = 11.19, F = 1.58, P = 1.18 > 0.05], and [Three months after the intervention: Box's M = 10.64, F = 1.36, P = 1.02 > 0.05]. The significance of the Box's test was more than 0.05; therefore, it is concluded that the variance–covariance matrices are homogeneous.

To compare the mean changes of cognitive health components of the elderly over time (three test stages), the repeated measures ANOVA method and SPSS v19 software were used. In all analyses, the effect of the initial value of variables and the effect of time were considered. The level of significance considered in this study is  $\alpha = 0.05$ .

## Results

#### **Baseline characteristics**

The mean age of study participants was  $70.03 \pm 5.42$  years, ranging from 60 to 80 years, as well as the mean body mass index (BMI) of study participants was  $22.65 \pm 4.21$ . Table 1 summarizes the baseline demographic and clinical characteristics.

The results of the General Health Questionnaire (GHQ-2) and Mini-Mental State Examination (MMSE) are specified in Table 2.

#### GHQ-2 test subscale scales

Table 2 illustrates the mean and standard deviation of GHQ-2 test subscale scores (depression, anxiety, physical symptoms, social performance, the whole) at 3 various stage tests. Table 3 shows an overall significant difference between the means at the 3 various stages. In the analysis of GHQ-2 subscales, all the main effects in time or stages were significant (depression ( $F_{(1.6)}$ =15.58 and  $P \le 0.001$ ), anxiety ( $F_{(2)}$ =340.43 and  $P \le 0.001$ ), physical symptoms ( $F_{(2)}$ =189.27 and  $P \le 0.001$ ),

Table 1 Baseline demographic and clinical characteristics

Scale	Age	Height	Weight	BMI
Mean	70.03	171.45	68.32	22.65
Std	5.42	4.29	9.46	4.21

Table 2Descriptive statisticsof quantitative variables of theGHQ-2 test

Scale	Ν	Initial evaluation $(M \pm SD)$	Two weeks after the intervention $(M \pm SD)$	Three months after the intervention $(M \pm SD)$
Depression	42	$8.589 \pm 1.660$	$7.580 \pm 1.431$	$8.248 \pm 1.165$
Anxiety	42	$13.777 \pm 2.448$	$11.733 \pm 2.348$	$12.622 \pm 2.292$
Physical symptoms	42	$12.656 \pm 1.359$	$11.622 \pm 2.292$	$11.628 \pm 2.305$
Social performance	42	$15.050 \pm 2.464$	$14.414 \pm 2.278$	$14.680 \pm 2.414$
Overall	42	$47.750 \pm 1.550$	$44.792 \pm 1.757$	$46.889 \pm 1.246$

Scores < 6 on each scale and scores < 22 on the whole test score indicate pathological symptoms

 Table 3 Results of the factorial analysis of variance repeated measures (ANOVARM) in estimating the effect of cognitive-motor training on general health

Scale	Type III sum of squares	df	F	Sig	Partial eta squared
Depression	22.115	1.619	15.587	0.001	0.312
Anxiety	88.236	2	340.433	0.001	0.893
Physical symptoms	40.481	2	189.275	0.001	0.822
Social performance	8.582	1.519	28.522	0.001	0.410
The whole	194.411	2	67.238	0.001	0.621

Significance level  $P \le 0.05$ 

social performance ( $F_{(1.5)} = 28.52$  and  $P \le 0.001$ ), and overall score ( $F_{(2)} = 67.23$  and  $P \le 0.001$ )) (Table 3).

Then, using the modified Bonferroni paired comparisons test, significant differences in depression scores between baseline assessment and short-term follow-up (MD = 1.009and P = 0.001) and also between short-term follow-up and long-term follow-up (MD = 0.668 and P = 0.001) were found. There were significant differences in anxiety scores between baseline assessment and short-term follow-up (MD = 2.044 and P = 0.001), baseline assessment and longterm follow-up (MD = 1.155 and P = 0.001), and short-term follow-up and long-term follow-up (MD = -0.889 and P = 0.001). There were significant differences in physical symptom scores between baseline assessment and shortterm follow-up (MD = 1.322 and P = 0.001), baseline assessment and long-term follow-up (MD = 1.028 and P = 0.001), and short-term follow-up and long-term followup (MD = -0.294 and P = 0.001). There were significant differences in social performance scores between baseline assessment and short-term follow-up (MD = 0.636 and P = 0.001), baseline assessment and long-term follow-up (MD = 0.370 and P = 0.001), and short-term follow-up and long-term follow-up (MD = -0.266 and P = 0.001). Also, there were significant differences in the overall scores between baseline assessment and short-term follow-up (MD = 2.958 and P = 0.001), baseline assessment and longterm follow-up (MD = 0.860 and P = 0.006), and short-term follow-up and long-term follow-up (MD = -2.097 and P = 0.001). However, there was not a significant difference in the depression scale between baseline assessment and long-term follow-up (MD = 0.340 and P = 0.082).

Figure 2(a) and (b) show the average scores of GHQ-2 test subscale scales of research for test various stages.

#### MMSE test subscale scales

Table 4 illustrates the mean and standard deviation of MMSE test subscale scores (orientation, information encoding, attention and calculation, recall, lingual skill, action performance) and the overall score at three various stages of the test. The differences were significant for attention and calculation ( $F_{(1.5)}$ =68.87 and  $P \le 0.001$ ), recall ( $F_{(2)}$ =18.07 and  $P \le 0.001$ ), lingual skill ( $F_{(2)}$ =36.23 and  $P \le 0.001$ ), action performance ( $F_{(2)}$ =57.74 and  $P \le 0.001$ ), and the overall score ( $F_{(1.6)}$ =83.56 and  $P \le 0.001$ ). Differences for orientation ( $F_{(1.1)}$ =3.79 and P=0.149) and information encoding ( $F_{(2)}$ =0.45 and P=0.636) scales were not significant (Table 5).

Then, using the modified Bonferroni paired comparisons test, it was found that there is a significant difference in orientation scale between baseline assessment and shortterm follow-up (MD = -0.441 and P = 0.001). Also, there were significant differences in attention and calculation scale between baseline assessment and short-term followup (MD = -0.757 and P = 0.001), baseline assessment and long-term follow-up (MD = -0.330 and P = 0.001), and short-term follow-up and long-term follow-up (MD = 0.427and P = 0.001). Also, there were significant differences in recall scores between baseline assessment and short-term follow-up (MD = -0.244 and P = 0.001), and baseline assessment and long-term follow-up (MD = -0.330 and P = 0.001). Also, there were significant differences in lingual skill score between baseline assessment and shortterm follow-up (MD = -0.343 and P = 0.001), and shortterm follow-up and long-term follow-up (MD = -0.270and P = 0.001). Also, there were significant differences in action performance score between baseline assessment and short-term follow-up (MD = -0.370 and P = 0.001), baseline assessment and long-term follow-up (MD = -0.796and P = 0.001), and short-term follow-up and long-term Table 4Descriptive statisticsof quantitative variables of theMMSE test

Scale		Initial evaluation $(M \pm SD)$	Two weeks after the intervention $(M \pm SD)$	Three months after the intervention $(M \pm SD)$
Orientation	42	$9.240 \pm 2.399$	$9.681 \pm 2.237$	$9.389 \pm 1.698$
Information encoding	42	$2.603 \pm 0.267$	$2.647 \pm 0.119$	$2.636 \pm 0.248$
Attention and calculation	42	$3.557 \pm 0.248$	$4.314 \pm 0.415$	$3.887 \pm 0.106$
Recall	42	$2.441 \pm 0.181$	$2.684 \pm 0.213$	$2.628 \pm 0.148$
Lingual skill	42	$2.538 \pm 0.218$	$2.881 \pm 0.160$	$2.611 \pm 0.223$
Action performance	42	$4.500 \pm 1.138$	$4.871 \pm 1.110$	$4.696 \pm 1.199$
Whole	42	$17.993 \pm 3.311$	$22.883 \pm 3.286$	$19.714 \pm 2.218$

The maximum score in each scale is 10 for orientation, 3 for information encoding, 5 for attention and calculation, 3 for recall, 3 for lingual skill, 5 for action performance, and 30 for the overall score. The overall score of 24–30 indicates normalcy and > 23 indicates the possibility of a disorder

**Table 5** Results of the factorial analysis of variance repeated measures (ANOVARM) in estimating the effect of cognitive-motor training on cognitive health

	Type III sum of squares	df	F	Sig	Partial eta squared
Orientation	4.230	1.113	3.799	0.149	0.050
Information encoding	0.044	2	0.455	0.636	0.011
Attention and calcu- lation	12.104	1.495	68.872	0.001	0.627
Recall	1.365	2	18.069	0.001	0.306
Lingual skill	2.746	2	36.236	0.001	0.469
Action performance	2.904	2	57.741	0.001	0.582
Whole	516.735	1.654	83.560	0.001	0.671

Significance level  $P \le 0.05$ 

follow-up (MD = 0.175 and P = 0.001). Also, there were significant differences in overall scores between baseline assessment and short-term follow-up (MD = -4.890 and P = 0.001), baseline assessment and long-term follow-up (MD = -1.721 and P = 0.001), and short-term followup and long-term follow-up (MD = 3.169 and P = 0.001). But there were no significant differences in the orientation scale between baseline assessment and long-term follow-up (MD = -0.149 and P = 0.782), and short-term follow-up and long-term follow-up (MD = 0.292 and P = 0.830). Also, there were no significant differences in information encoding scale between baseline assessment and short-term followup (MD = -0.044 and P = 0.092), baseline assessment and long-term follow-up (MD = -0.033 and P = 0.152), and short-term follow-up and long-term follow-up (MD = 0.011 and P = 0.425). Also, there was not a significant difference in information recall scale between short-term followup and long-term follow-up (MD = 0.057 and P = 0.560). Also, there was not a significant difference in lingual skill scale between baseline assessment and long-term follow-up (MD = -0.073 and P = 0.422).

Figure 3(a) and (b) show the average scores of MMSE test subscale scales of research for at test various stages.

# Discussion

The objective of the present study was to investigate the effect of cognitive-motor training on reconstructing cognitive and mental health components in older adults who recovered from COVID-19. The elderly subjects who recover from COVID-19 show pathological symptoms related to cognitive and mental health. Meanwhile, participation in the CMT twice a week could reconstruct almost all domains of cognitive functioning. Even though findings need to be interpreted carefully because of the design under the influence of COVID-19 quarantine situation, it seems that a training program composed of CMT would be beneficial to prevent cognitive health decline in older adults who recovered from COVID-19.

In the present study, performing combined cognitivemotor training made it possible for the elderly to perform motor tasks such as balance training along with cognitive task at the same time, which causes simultaneous involvement of motor and cognitive systems (Fig. 4). This led to improved cognitive abilities and a proper division of attention between tasks [27].

The types of exercises were chosen completely in line with the corporate health protocols related to COVID-19 and quarantine procedures, with the aim of increasing the cognitive health of the elderly. The closer the participants got from the first session to the last session of the exercises, the harder the exercises became and the more effort the person had to put in the tasks. As a result, this type of exercise helped improve the cognitive health of the elderly.

These findings are consistent with studies indicating CMT makes that improving in cognitive health of elderly people [15, 28, 29]. In CMT, two or more cognitive-motor tasks are simultaneously performed. In general, cognitive tasks, such



**Fig.3** The effects of cognitive-motor training on cognitive health ((a) orientation, (b) information encoding, (c) attention and calculation, (d) recall, (e) lingual skill, (f) action performance, and (g) the whole) at test various stages. \*Significance level  $P \le 0.05$ 

Fig. 4 The cognitive-motor training scenario considered in this study (The training program used progressive activities related to body stability, to body stability plus hand manipulation, then body transport, and finally body transport plus hand manipulation. The participants receiving dual-task training with fixed-priority instructions practiced motor tasks while simultaneously performing cognitive tasks, and were instructed to maintain attention on both postural and cognitive tasks at all times.)



as calculation or word retrieval, are often performed during motor tasks, such as walking or stepping. Some studies in line with our results have demonstrated that CMT improved cognitive health and this training was more effective than single-task training in improving various cognitive domains [15, 28]. Eggenberger and colleagues (2015) have demonstrated that multicomponent physical exercise with simultaneous cognitive training boosts cognitive performance in older adults [30]. Morita and colleagues (2018) have demonstrated that participating in exercise program comprising cognitive-motor dual-task training may be beneficial for maintaining the broad domains of cognitive function (such as attention and the total score of the 3MS examination) in healthy elderly people [15].

In the present study, the training program composed of CMT prevented deterioration in the cognitive and mental disorders of older adults recovering from the COVID-19, with mean age of 70 years.

It seems CMT, similar to physical activity or exercise, induces alterations at the cellular and molecular levels, which is likely to initiate structural and functional adaptations in the brain, and/or behavioral/socio-emotional changes that eventually influence cognitive health. Moreover, it is possible that CMT, through influencing the neurophysiological mechanisms, causes reconstructing cognitive and mental health components in older adults. In explaining this finding, it can be said that CMT may cause increase in cerebral blood flow [31] and angiogenesis [32] to improve cognitive health. In this regard, Ohsugi et al. (2013) reported that CMT significantly increased blood flow and the activity assessed by the quantity of oxygenated hemoglobin in the prefrontal cortex, the primary brain area that exerts executive function [33].

Therefore, the favorable effect of CMT training on reconstructing cognitive and mental health may be, at least in part, increase in cerebral blood flow.

The authors believe that if the older adults are encouraged to perform these exercises routinely and for a long period of time, these exercises will have a greater impact on their cognitive health components. Their cognitive function in turn will have a positive impact on mental conditions and social functioning and thus improve their total health. Therefore, this low-cost, effective training should be used by health care providers for reconstructing cognitive health of patients.

However, due to the limited population studied in this investigation, further studies are needed to make the results more stable about the effect of CMT on cognitive and mental health.

It is also necessary to compare the effect of this training method with other methods to recognize the most appropriate training method for reconstructing and promoting cognitive and mental health components in older adults recovering from COVID-19. Meanwhile, determining the ideal CMT prescription that targets motor performance and cognitive ability should be considered.

The present study has several limitations: First, the current COVID-19 quarantine situation in the community led to the use of an intra-group design with a small sample size. Second, to date, appropriate exercise frequency, intensity, and duration for elderly people with cognitive and mental impairment have not been carefully established.

# Conclusion

Therefore, authors, consulting the experts, designed a training program to the best of their knowledge and experience, which needs to be supported from other investigators. A third limitation of the present study was that the training program was performed only twice a week. This was done because of limitations in the participant's ability to follow the exercise protocol. For a better result in improving the health of the elderly, 3–5 exercise sessions a week will be necessary [34]. Finally, the MMSE, used in the present study, is mainly used for screening

purposes, not to measure the effectiveness of an intervention. This limitation may also limit the generalizability of the results.

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**Data availability** All original data will be available upon contact with the corresponding author.

## Declarations

Human and animal rights The present study followed the ethical standards for human and animal rights.

**Ethical approval** The study protocol has been approved by the Regional Research Ethics Committee in Baqiyatallah University of Medical Sciences (reference IR.BMSU.REC.1399.392).

**Consent to participate** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (national and institutional). Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

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