#### ORIGINAL ARTICLE



# The Effect of Whole Body Vibration on Postural Control of Ataxic Patients: a Randomized Controlled Cross-Over Study

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## Abstract

Whole body vibration (WBV) applications have been used in recent years to increase muscle strength, power, and postural control in healthy and various disease populations. This study aims to investigate the effects of WBV on postural control in patients with ataxia. Twenty-four patients were randomly allocated to two groups. In the first group, whole body vibration and exercise therapy (WBV + E) were applied together for the first 8 weeks; after 1 week washout, only exercise program (OE) was applied for the second 8 weeks. In the second group, the OE program was applied first followed by the WBV + E program. Outcome measures were Sensory Organization Test (SOT), Adaptation Test (ADT), Limits of Stability Test (LOS), International Classification Ataxia Ratio Scale (ICARS), Berg Balance Scale (BBS), and Timed Up and Go Test with cognitive task (TUG-C). Twenty patients (mean age  $\pm$  SD, 34.00 $\pm$ 9.16 years) completed the study. The scores of SOT, ICARS, and BBS improved significantly after both OE and WBV + E program ( $p < 0.05$ ). Improvements in the WBV + E program were higher ( $p < 0.05$ ). The scores of ADT, TUG-C, and three parameters of LOS improved significantly after WBV + E ( $p < 0.05$ ), while there was no significant change after OE ( $p > 0.05$ ). This study demonstrated that exercise programs supported by WBV can play an important role in the improvement of all components of postural control in patients with ataxia. ClinicalTrial.gov Identifier: NCT02977377

Keywords Ataxia . Exercise therapy . Whole body vibration . Postural control . Rehabilitation

# Introduction

Ataxia is a clinical syndrome in which muscular incoordination emerges due to dysfunction in the cerebellum and neural connections [\[1](#page-7-0)]. Balance and coordination impairments, walking difficulty, loss of hand skills, and speech impairment are the most important findings of ataxia  $[1, 2]$  $[1, 2]$  $[1, 2]$ . Postural control

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deficits are at the forefront because equilibrium and walking problems are the most important underlying cause of falls in ataxic patients.

Horak [\[3\]](#page-7-0) noted six important resources for postural control: "sensory strategies", "movement strategies", "biomechanical constraints", "orientation in space", "control of dynamics", and "cognitive processing". A disorder in any one or a combination of these resources leads to postural instability [\[3](#page-7-0)]. Assessment of these six resources together in the evaluation of postural control gives the most accurate and detailed results.

Postural instability and gait ataxia are the most important findings of diseases with cerebellum damage, especially spinocerebellar ataxia and multiple sclerosis. Postural control loss in these diseases manifests itself in three ways in patients. The first of these is the difficulty in maintaining an upright position, the second one is the narrowing of the stability limits, and the third one is the delay in response to postural changes [\[4](#page-7-0)]. Despite the fact that development of postural control is one of the most important goals in ataxia rehabilitation, there are not enough studies in this regard [[5](#page-7-0)]. When the literature is examined, exercise therapy is the most important option in the

treatment of ataxic patients [[6,](#page-7-0) [7\]](#page-7-0). Although exercise therapy has positive effects on ataxic findings and postural control, different physiotherapy methods continue to be tried in this field due to chronic and prolonged progression of the disease [\[8\]](#page-7-0). One of these new methods is whole body vibration (WBV).

WBV applications have been used in recent years to increase muscle strength, power, and postural control in healthy and various disease populations [[9](#page-7-0)]. Studies investigating the efficacy of WBV in various neurological problems such as multiple sclerosis, Parkinson's disease, cerebral palsy, and stroke in the literature have shown some positive effects on treatment programs aimed especially at improving postural control  $[10-13]$  $[10-13]$  $[10-13]$ . But, there is a weak evidence for a positive effect of short-term WBV training on spasticity of lower limbs, mobility, balance, and postural control in patients with neurological disorders. Regarding the long-term effect of WBV, there is a weak evidence only for a positive effect on mobility in patients with neurological disorders [[14\]](#page-7-0). Even though ataxia is a neurological condition that affects postural control in many ways, no study has yet shown the efficacy of WBV in combination with an individualized exercise program in ataxic patients. The purpose of this randomized controlled study is to examine the effects of whole body vibration on postural control of ataxic patients.

# Materials and Methods

## **Participants**

This study was conducted in the Faculty of Physical Therapy and Rehabilitation of Hacettepe University. It was approved by the Ethics Committee of the Hacettepe University (GO 14/397). The inclusion criteria had four parameters:

- 1. Being diagnosed with cerebellar ataxia by a neurologist
- 2. Walking independently (without any help or assistive device)
- 3. Mini–Mental State Examination score  $\geq$  24 points [[15,](#page-7-0) [16](#page-7-0)]
- 4. Having an Expanded Disability Status Scale cerebellar system score  $\geq$  3 for ataxic multiple sclerosis patients [\[17\]](#page-7-0)

Exclusion criteria for the study included six parameters:

- 1. Predominantly vestibular and sensory ataxia symptoms and muscle weakness
- 2. Increased muscle tone of lower extremity muscles (Modified Ashworth Scale score  $\geq$  2)
- 3. Having an Expanded Disability Status Scale pyramidal functional system score > 3 for ataxic multiple sclerosis patients [[17\]](#page-7-0),
- 4. Systemic diseases and cognitive impairment
- 5. Communication problems
- 6. Other orthopaedic and neurological problems that may affect postural control

The patients who agreed to participate in the study were informed in detail about the study. The patients gave written informed consent as approved by the Ethics Committee.

## Design

The study was designed as a randomized controlled, assessorblinded, cross-over trial. Randomization was performed using a computer program, and patients were randomly allocated to two groups via the program. In the first group, a whole body vibration and exercise (WBV  $+ E$ ) program were applied together for the first 8 weeks; after 1 week washout period, only exercise program (OE) was applied for the second 8 weeks.

In the second group, an OE program was applied for the first 8 weeks, and after 1 week washout period, WBV + E program was applied for the second 8 weeks. The flow chart of the study is shown in Fig. [1.](#page-2-0) All randomization process and trainings were performed by the same physiotherapist who was not blinded to group allocation.

## Outcome Measures

The assessments were applied four times before and after both treatments by the same physiotherapist who was blind to the study. Demographic information such as age, gender, body weight, height, and type of diagnosis were recorded before the first assessment. Posturography assessments and other clinical assessments were carried out in two different sessions to prevent fatigue. Also, these evaluations were applied randomly to prevent the learning effect.

#### Posturography Assessments

Sensory Organization Test (SOT) The Computerized Dynamic Posturography (Neurocom Smart Balance Master System Inc., Clackamas, OR) consists of a combination of various tests evaluating different aspects of postural control. In this study, Sensory Organization Test of Computerized Dynamic Posturography was used to assess "sensory strategies" component of postural control. The functions of the somatosensory, vestibular, and visual systems contributing to postural control are objectively determined in six test conditions in the SOT. These six conditions are established using visual environmental changes or platform rotations when the patient's eyes are open/closed. The percentage of the equilibrium called the composite score—is obtained using six test conditions in the SOT [\[18,](#page-7-0) [19](#page-7-0)]. The SOT has also been applied as a

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Fig. 1 Flow chart of the study

gold standard for the validation of novel outcome measures of postural control [[20\]](#page-8-0).

Adaptation Test (ADT) ADT assesses the ability to adapt somatosensory input that emerges according to an unexpected change in the patient's support surface orientation. During the test, the platform is first antero-posteriorly rotated suddenly to lift the patient's toes up for 5 times; the toes are then moved down 5 times [\[21](#page-8-0)]. The ADT was used to assess the "movement strategies" component of the postural control.

Limits of Stability Test (LOS) The Limits of Stability Test of Computerized Dynamic Posturography evaluates the ability to control the movement of the gravity to centre over the support surface. The voluntary control of the gravity centre is achieved by asking the patient to shift their weight in 8 different directions. In each direction, the reaction time (RT), directional control (DCL), endpoint excursion (EPE), movement velocity (MVL), and maximum excursion (MXE) parameters were

evaluated [[22\]](#page-8-0). The LOS was used to assess the "biomechanical constraints" component of the postural control.

#### Severity of Ataxia

The International Cooperative Ataxia Rating Scale (ICARS) was used to assess severity of ataxia and consists of four subscales: posture and gait disturbances, kinetic functions, speech disorders, and oculomotor disorders [[23](#page-8-0)]. The ICARS was used to assess the "control of dynamics" component of the postural control.

### Performance-Based Balance

The performance-based balances of the individuals were assessed with the Berg Balance Scale (BBS). The BBS includes scoring between 0 (not applicable) and 4 (normal performance) for the performance of 14 different tasks [[24,](#page-8-0) [25\]](#page-8-0).

The BBS was used to assess the "orientation in space" component of the postural control.

#### Functional Mobility with Dual Task

The Timed Up and Go Test (TUG) was used to assess functional mobility of the individuals. Then the test was repeated by adding a cognitive additional task. The cognitive task was applied in the form of a 3-by-3 count back from 100 [[26](#page-8-0)]. TUG tests were used to assess the "cognitive processing" component of the postural control.

## Interventions

The participants were randomized into two groups and entered into the physiotherapy program for 16 weeks (8 weeks\*2): 3 days a week for 1 h per day. Interventions were performed by 2 physiotherapists.

#### Whole Body Vibration

WBV was applied via the Compex<sup>®</sup> Winplate (Chattanooga) in 4 sets as 1-min application and 1-min rest. WBV was applied with 30 Hz and low amplitude (2 mm) as vertical oscillations. The parameters were chosen by analysing the studies examining the effects of WBV on postural control in the literature [\[27](#page-8-0)].

During the application, the individuals were asked to maintain a static posture in which the legs were held in a slightly flexed (slight squat position) with the feet open at the shoulder width.

#### Exercise Program

The individual needs of the patients were considered when the exercise programs were determined from trunk stabilization, balance, and functional exercises. The main objectives of the exercise programs are to improve trunk and proximal extremity stabilization, to develop balance and postural reactions against external stimuli and gravity, to improve functions of the extremities, to provide functional gait, and to increase independence.

The degree of difficulty of the exercises was determined in accordance with patients' individual performances. A treatment session was created with a 60-min exercise program. Exercise programs applied to the individuals were generally chosen from the following exercise approaches:

- I. Mat activities (with approximations from Proprioceptive Neuromuscular Facilitation techniques in different conditions)
- II. Static and dynamic balance exercises (with rhythmic stabilization and stabilizing reversal from Proprioceptive

Neuromuscular Facilitation techniques in different conditions)

- III Frenkel coordination exercises (with sensory stimulation techniques)
- IV Weight transfer and walking training on different kinds of ground (walking on narrow line, tandem walking, walking with excessive hip and knee flexion, walking on different kinds of ground, stopping and turning with sudden commands)
- V. Functional activities (sit to stand-stand to sit, climbing up and down stairs, ball activities while standing upright)

In the 5th week, exercises were updated according to the current condition of each patient. For example, the level of the Frenkel coordination exercises was improved, or the chair height was lowered while performing sit-to-stand activity.

#### Statistical Analysis

Statistical analyses were performed using the SPSS software package (version 20.0, SPSS Inc., Chicago, IL). The quantitative data were expressed as median (25–75% interquartile range) (median (IQR)). The normal distribution of the obtained data was evaluated visually (histogram and probability plots) and by using the Kolmogorov–Smirnov/Shapiro–Wilk tests. Nonparametric tests were used because the data obtained were not normally distributed.

Since the study was planned as a cross-over design and the data did not show normal distribution, first of all, the Mann– Whitney U test was used to compare baseline assessments of two groups, and then the difference between the periods was evaluated with the Mann–Whitney U test to see the period effect. The Wilcoxon two-tailed paired test was used to compare the efficacy of treatment programs, between baseline and after treatments; the level of significance was set at  $p < 0.05$ .

# Results

The study began with 24 patients and completed with 20. The demographic information of the patients is given in Table [1](#page-4-0).

There was no statistically significant difference at baseline assessment scores of two groups  $(p > 0.05)$  (Table [2\)](#page-4-0). In order to examine the effect of applying either of the two exercise approaches before or after, period effect was examined. The group, which WBV + E program was applied first, followed by the OE program, was named as "period I", and the group which OE was applied first and then WBV + E was called "period II". At the end of analysis, there was no statistically significant difference in all of the parameters evaluated  $(p > 0.05)$  (Table [3](#page-5-0)).

In the study, since all parameters had both no difference at baseline assessments and no period effect, the data of the

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 $*p$  < 0.05 Mann–Whitney U test

groups were combined, and subsequent analyses were done accordingly.

After both OE and WBV  $+ E$  program, the composite score of SOT improved significantly  $(p < 0.05)$  (Table [4\)](#page-5-0). When the two groups were compared, change after  $WBV + E$  was higher than that after OE ( $p < 0.05$ ) (Table [5\)](#page-6-0). The toes up and down scores of the Adaptation Test improved only after WBV + E  $(p < 0.05)$  (Table [4\)](#page-5-0). While there was no change in the RT and MVL parameters of the LOS test after WBV + E, the EPE, MXE, and DCL parameters improved significantly  $(p < 0.05)$  (Table [4](#page-5-0)).

ICARS scores improved significantly after both treatment programs ( $p < 0.05$ ) (Table [4\)](#page-5-0). The improvement after WBV + E was more than the OE program  $(p < 0.05)$ (Table [5\)](#page-6-0). The BBS score improved significantly after both treatments ( $p < 0.05$ ) (Table [4\)](#page-5-0). The change after WBV + E program was higher than the OE program  $(p < 0.05)$ (Table [5](#page-6-0)). There were significant improvements in both TUG and TUG with additional cognitive tasks after WBV + E  $(p < 0.05)$  (Table [4\)](#page-5-0). A cognitive task-based difference ((TUG-C)-(TUG)) was calculated to determine the burden of the cognitive task in the TUG with additional cognitive task. The increase in time that was caused by the additional cognitive task decreased only after WBV + E  $(p < 0.05)$  (Table [4\)](#page-5-0).

The study power was calculated from Gpower 3.0.10 analysis program. The Sensory Organization Test results were used to determine the power of the study. Post treatment means, standard deviations, and sample sizes of groups were used to calculate the achieved power. A power of 81.4% was obtained.



Table 2 The difference between baseline scores of groups

 $*p$  < 0.05 Mann–Whitney U test

SOT Sensory Organization Test, ADT Adaptation Test, LOS\_RT Limits of Stability Test reaction time, LOS\_MVL Limits of Stability Test movement velocity, LOS\_EPE Limits of Stability Test endpoint excursion, LOS\_MXE Limits of Stability Test maximum excursion, LOS\_DCL Limits of Stability Test directional control, ICARS International Cooperative Ataxia Rating Scale, BBS Berg Balance Scale, TUG Timed Up and Go Test, TUG-C Timed Up and Go Test with cognitive task

<span id="page-5-0"></span>Table 3 Comparisons of the groups' period effects



 $*p$  < 0.05 Mann–Whitney U test

SOT Sensory Organization Test, ADT Adaptation Test, LOS\_RT Limits of Stability Test reaction time, LOS\_MVL Limits of Stability Test movement velocity, LOS\_EPE Limits of Stability Test endpoint excursion, LOS\_MXE Limits of Stability Test maximum excursion, LOS DCL Limits of Stability Test directional control, ICARS International Cooperative Ataxia Rating Scale, BBS Berg Balance Scale, TUG Timed Up and Go Test, TUG-C Timed Up and Go Test with cognitive task

# **Discussion**

This study investigated the effects of whole body vibration on postural control in patients with ataxia. The most important finding was meaningful improvements in all components of postural control when the individualized exercise program was supported with whole body vibration. On the other hand, limited improvements in only some components of postural

Table 4 Results of posturography and clinical evaluations of the interventions

	Only exercise program $(n:20)$				Whole body vibration and exercise program $(n:20)$			
	Pre-treatment Median (IQR)	Post-treatment Median (IQR)	Z	$\boldsymbol{p}$	Pre-treatment Median (IQR)	Post-treatment Median (IQR)	Z	$\boldsymbol{p}$
SOT $(0-100)$	$66.50(51.25 - 80.25)$	$67.50(61.00-79.00)$	2.942	$0.003*$	59.50 (45.00–70.75)	77.00 (68.00–83.00)	3.734	$0.000*$
ADT Toes Up $(0 - 200)$	$72.00(59.25 - 88.75)$	69.00 (64.00–75.50)	$-1.107$	0.268	71.50 (57.50-83.00)	$65.00(56.50 - 80.75)$	$-2.136$ 0.033*	
ADT Toes Down $(0 - 200)$	55.50 (53.50–67.25)	$60.00(47.00-77.75)$	0.524	0.601	$62.50(56.25 - 85.50)$	$55.00(47.25 - 60.50)$	$-3.439$ 0.001*	
LOS_RT	$0.89(0.74 - 1.08)$	$0.95(0.78-1.09)$	$-0.056$ 0.955		$1.05(0.78-1.26)$	$0.85(0.74 - 1.07)$	$-1.605$ 0.108	
LOS_MVL	$4.16(3.16-4.82)$	$3.63(2.95-4.30)$	$-1.008$ 0.313		$3.63(2.32 - 4.16)$	$3.96(3.54 - 4.56)$	$-1.941$	0.052
LOS EPE	74.13 (57.63-77.44)	$68.50(61.50-76.00)$	$-0.322$ 0.748		62.44 (49.50-71.06)	76.69 (71.44–82.19)	$-3.662$ 0.000*	
LOS MXE	90.38 (82.03–93.25)	89.50 (79.75-96.53)	0.037	0.970	82.50 (67.53-90.63)	93.06 (89.41-95.44)	3.509	$0.000*$
LOS_DCL	75.13 (71.47-79.31)	79.44 (60.34–81.56)	$-0.112$	0.911	64.75 (56.09-78.44)	79.38 (75.25-81.31)	$-3.528$	$0.000*$
ICARS $(0-100)$	$10.00(7.00-16.25)$	$8.00(6.00-17.50)$	$-3.248$	$0.001*$	$11.00(9.00-22.50)$	$7.50(5.00-14.00)$	$-3.933$	$0.000*$
BBS (0-56)	52.50 (47.00-54.00)	52.50 (49.00–55.00)	2.491	$0.013*$	49.00 (47.25 - 51.00)	53.00 (52.00-55.00)	3.734	$0.000*$
TUG	$8.02(6.92 - 11.22)$	$8.18(7.07-10.22)$	$-0.765$	0.444	$8.73(7.20 - 10.23)$	$8.02(6.53 - 10.00)$	$-3.472$	$0.001*$
TUG-C	$9.23(7.63 - 12.23)$	$9.13(7.98 - 10.97)$	$-0.075$	0.940	$9.80(7.99 - 11.26)$	$9.20(7.22 - 11.01)$	$-2.558$ 0.011*	
$(TUG-C)-(TUG)$	$0.77(0.54-1.71)$	$0.99(0.50-1.61)$	$-0,747$ 0.455		$1.13(0.76-1.84)$	$0.73(0.42 - 1.31)$	$-3.846$ 0.000*	

 $*p$  < 0.05 Wilcoxon two-tailed paired test

SOT Sensory Organization Test, ADT Adaptation Test, LOS\_RT Limits of Stability Test reaction time, LOS\_MVL Limits of Stability Test movement velocity, LOS\_EPE Limits of Stability Test endpoint excursion, LOS\_MXE Limits of Stability Test maximum excursion, LOS\_DCL Limits of Stability Test directional control, ICARS International Cooperative Ataxia Rating Scale, BBS Berg Balance Scale, TUG Timed Up and Go Test, TUG-C Timed Up and Go Test with cognitive task

<span id="page-6-0"></span>Table 5 Changes in SOT, ICARS, and BBS scores, between exercise programs comparisons



 $*p$  < 0.05 Wilcoxon two-tailed paired test

SOT Sensory Organization Test, ICARS International Cooperative Ataxia Rating Scale, BBS Berg Balance Scale

control were observed with an individualized exercise program applied alone for 8 weeks.

"Sensory strategies" is an important component of postural control, and objective analysis of sensory strategies was recommended in the first steps of treatment [\[28,](#page-8-0) [29\]](#page-8-0). Two separate studies examined the acute effect of WBV on balance in patients with multiple sclerosis (a session and five sessions of WBV application); the researchers observed an increase in the composite score of SOT, but they could not statistically show the difference in the results [[10](#page-7-0), [30](#page-8-0)]. The SOT composite score did not improve in previous studies, but it increased significantly after both treatments in the present study. The meaningful improvement seen in the study was likely because of the 8-week treatment that was sufficient to develop the sensory system  $[31]$ . Further improvements in the WBV + E group indicates that when WBV is added to the exercise program, sensory integration and the ability to use sensory systems in postural control increase according to the exercise program applied alone.

Postural control requires adapting to changing tasks and environmental needs. This is related to multiple action strategies and the ability to choose the appropriate strategy for the task/environment [[32](#page-8-0)]. Improvements in both direction swing scores in the ADT test were obtained after the WBV  $+ E$ , while there was no change after OE. These results suggest that individualized exercise program that aimed to improve adaptive postural reactions (dynamic balance exercises such as throwing-catching a ball and sudden turns) are inadequate when it is applied alone. In contrast, when exercise therapy is supported by WBV, there is an increase in muscular activity, co-contraction, and also improved somatosensory input adaptability in relation to the development of sensory organization [\[33](#page-8-0)].

Although LOS is clinically important, only one study used the test to investigate the effectiveness of WBV. Cheung et al. applied WBV without any exercise for 3 days/week over 3 months in geriatric individuals and found improvements in DCL, MXE, and MVL parameters [[34\]](#page-8-0). In the present study, improvements were observed in MXE, DCL, and EPE after  $WBV + E$ . We thought that the application of the individual exercise training and WBV together allowed the patients to gain more than Cheung's study. Also we aimed to stimulate slower and more controlled movements in this study because of the disorder of voluntary co-contractions of agonist/ antagonist postural muscles and affected corrective movements involving the whole body in ataxia [[35\]](#page-8-0). So it can be considered a desired result that no change in the velocity and the reaction time parameters after treatment.

In previous studies evaluating the efficacy of WBV, only one study evaluated severity of ataxia as a component of the "control of dynamics". Kaut et al. used ICARS in order to evaluate the effect of WBV (application for 4 days) in patients with spinocerebellar ataxia, but they could not show the statistical improvement [[36](#page-8-0)]. In the present study, the decrease in severity of ataxia after both 8-week exercise programs was related to the duration and quality of the exercise programs. The decrease in severity of ataxia by exercise was expected, while advances in favour of  $WBV + E$  were likely caused by activation of joint mechanoreceptors and stimulation of gamma efferents.

In a study examining the effect of WBV in patients with MS for 5-day application, no improvement was observed in the performance-based balance [\[30](#page-8-0)]. Similarly, other studies examined the effect of WBV application with a standard exercise program that was not specific to the individuals for 3– 20 weeks in MS patients; there were no improvements in the performance-based balance [[37](#page-8-0), [38\]](#page-8-0). Our results differ from the literature and were likely due to exercise programs developed for individual needs and neuromuscular activation resulting from WBV application which increased the effect of these exercises and proprioceptive inputs.

The performance of postural tasks can be impaired by secondary cognitive task because postural control and other cognitive tasks share cognitive resources [\[39\]](#page-8-0). In this study, the improvement of functional mobility skills involving dual tasks after WBV shows that individuals reduce the need for attention and that they transfer attention to postural control due to improvements in other components of postural control.

One important limitation of this study was that it was only single blinded. In addition to the blind physiotherapist, an exercise program with sham WBV application could be done. However, in this case, a third exercise program was not <span id="page-7-0"></span>preferred in the study design because the duration of the study would be long, and we did not want to compromise the study methodology. Although the exact time has not been indicated in the literature for removing the effects of the balance exercises, 1-week washout period might be one of the limitations of the present study.

# Conclusions

This study is the first with a high level of evidence to evaluate the contribution of WBV to an exercise program that improves postural control in patients with ataxia. The addition of WBV to the exercise program leads to significant gains in all components of postural control with an increase in sensory organization abilities, limits of stability, and muscular coordination. In the light of this evidence, we concluded that WBV is suitable for clinical use because of the ease of administration in the rehabilitation of patients with ataxia in which all components of postural control are affected.

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Authors' Contributions Ender Ayvat: Conceptualization, data curation, formal analysis, methodology, writing-original draft, and writing-review and editing. Muhammed Kılınç: Conceptualization, data curation, formal analysis, methodology, writing-original draft, and writing-review and editing. Fatma Ayvat: Data curation, formal analysis, methodology, and writing-review and editing. Özge Onursal Kılınç: Data curation, formal analysis, methodology, and writing-review and editing. Sibel Aksu Yıldırım: Conceptualization, formal analysis, methodology, and writing-review and editing.

## Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval The study was approved by the Ethics Committee of the Hacettepe University (GO 14/397) and all procedures performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent to Participate Before starting the experiment, all participants were informed about objectives and methods of the study and asked to sign informed consent.

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