Turk J Phys Med Rehab 2023;69(2):222-229
DOI: 10.5606/tftrd.2023.11990
Available online at www.turkishjournalpmr.com

Original Article

The relationship of multifidus and gastrocnemius muscle thickness with postural stability in patients with ankylosing spondylitis

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Received: October 27, 2022 **Accepted:** December 20, 2022 **Published online:** March 31, 2023

ABSTRACT

Objectives: This study aimed to investigate potential changes in the thickness of the multifidus and gastrocnemius muscles and to demonstrate the association of muscle thickness with postural stability in ankylosing spondylitis (AS) patients.

Patients and methods: The cross-sectional observational study enrolled 32 AS patients (23 males, 9 females; mean age: 39.4±7.2 years; range, 18 to 65 years) diagnosed according to the modified New York criteria and 32 healthy controls (22 males, 10 females; mean age: 36.6±7.5 years; range, 18 to 65 years) between April 2017 and October 2018. Plantar center of pressure (CoP) excursions were recorded using a pressure platform to evaluate postural stability. The thickness of the lumbar multifidus and gastrocnemius muscles was measured using ultrasound.

Results: Patients with AS showed reduced muscle thickness at the multifidus ($p<0.05$) muscle and medial gastrocnemius ($p=0.002$) and lateral gastrocnemius ($p=0.002$) muscles compared to controls. Increased CoP excursions were observed only in the anteroposterior direction in the double-leg (standard) stance with the eyes closed ($p=0.003$) and in both anteroposterior and mediolateral directions in tandem and single-leg stances (all $p<0.05$). Center of pressure excursions in standard stance with the eyes closed were negatively correlated with all muscle thickness values (all $p<0.05$). In the single-leg stance, CoP excursions were negatively correlated with muscle thickness of medial gastrocnemius ($p=0.008$) and lateral G ($p=0.016$) muscles.

Conclusion: Early planning of exercise programs taking muscle loss into account can help improve balance and thereby prevent falls and fractures in AS patients.

Keywords: Ankylosing spondylitis, multifidus, postural balance, ultrasonography.

Ankylosing spondylitis (AS) is a common chronic inflammatory rheumatic disease that primarily affects the axial skeleton. In addition to pain, the disease causes a variety of biomechanical problems due to structural abnormalities that develop in the musculoskeletal system over time. Patients with AS were reported to have poorer balance than healthy individuals.[1-3] It is known that balance is affected in many conditions and associated with chronic low back pain.[4] In AS patients, several factors have been associated with impaired balance, including poor posture, limitation of joint movements, and pain.[2,3,5,6] However, the factors involved in balance impairment in these patients are still unclear. It is known that AS patients have an increased prevalence of vertebral fractures. For this reason, it is important to identify the factors affecting balance impairment that may increase the risk of falls to prevent fractures.

Atrophy of the paraspinal muscles has been observed in patients with AS.[7] In a recent study,

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Cite this article as:
Mesci E, Mesci N. The relationship of multifidus and gastrocnemius muscle thickness with postural stability in patients with ankylosing spondylitis. Turk J Phys Med Rehab 2023;69(2):222-229. doi: 10.5606/tftrd.2023.11990.

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denervation of the paraspinal muscles was found to occur in AS patients.[8] Reduced muscle strength of some core muscles, such as the back extensor muscles, has been reported in AS patients compared to controls. Paraspinal muscle atrophy has been implicated as the possible cause of the muscle weakness.[3] In a separate study, an association was found between core muscle endurance times and balance in AS patients.[9] Additionally, weakness and premature fatigue of ankle dorsiflexor muscles were shown in patients with AS by isokinetic testing.[10]

Evidently, a significant amount of data on muscle denervation, atrophy, weakness, and performance in AS patients has been added to the literature in recent years. Both the core muscles, such as paraspinal muscles, and the lower extremity muscles, such as the gastrocnemius muscle, which controls ankle movements, have important roles in maintaining postural stability. It is known that denervation and loss of strength of the multifidus muscles impair postural control and balance.[11] Fatiguing the ankle dorsiflexor and plantar flexor muscles with isokinetic exercises has been shown to significantly impair postural stability.[12] There are new findings on the association of thickness of some lower limb and core muscles with balance in both young and elderly populations.[13]

In our review of the literature, no study was identified on the effects of lower limb or paraspinal muscle thickness on postural stability in AS patients. We aimed to investigate potential changes in the thickness of the multifidus and gastrocnemius muscles, both of which are crucial for postural stability, and to determine whether muscle thickness is associated with postural stability. Our study tested the hypothesis that patients with AS have thinner multifidus and gastrocnemius muscles compared to the healthy population and the reduced thickness of these muscles negatively affects the postural stability of the patients.

PATIENTS AND METHODS

This cross-sectional observational study conducted at the Istanbul Medeniyet University Göztepe Training and Research Hospital between April 2017 and October 2018. A total of 32 patients (23 males, 9 females; mean age: 39.4±7.2 years; range, 18 to 65 years) diagnosed with AS according to the modified New York criteria were included in this study. The control group consisted of 32 age-and sex-matched healthy individuals (22 males, 10 females; mean age: 36.6±7.5 years; range, 18 to 65 years). Patients with neurological disorders, diabetes, or orthopedic conditions, those with previous surgery to the spine or lower extremities, those with problems affecting vision or the vestibular system and subjects failing to complete the study tests were excluded from the study. Age, sex, body weight, height, and body mass index were recorded for the patient and control groups. The Bath Ankylosing Spondylitis Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), and Bath Ankylosing Spondylitis Metrology Index (BASMI) scores were obtained for AS patients.

The Timed Up And Go (TUG) test was used to assess dynamic balance. It measures the time a participant takes to stand up from a chair, walk a 3-m distance, and return and sit on the chair again. The TUG test has been demonstrated to be valid and reliable for the evaluation of clinical balance in rheumatologic diseases.[14]

Plantar center of pressure (CoP) excursions were measured for the assessment of postural stability. A Win-Track pressure platform (Medicapteurs Technology, Balma, France) was used for this purpose. Win-Track is a 150×50 cm device that measures vertical ground reaction force with 12,288 sensors on its pressure-sensitive surface. The first measurements were obtained with the patient on both feet (standard stance). While obtaining measurements in the standard stance, care was taken to allow a distance of 22, 26, and 30 cm between the feet of patients with a height of 76 to 140, 141 to 165, and 166 to 203 cm, respectively.[15] The second measurements were repeated in the tandem stance. Measurements for these postures were performed twice, with eyes open and closed. Lastly, CoP excursions were measured with the subject standing on the dominant leg and the eyes open. Care was taken to ensure the arms were held right beside the body during the measurements. Postural stability data were acquired by recording CoP excursions for 30 sec on the pressure platform. The total trajectory value, which indicates the lengths of trajectories in all directions, the anteroposterior trajectory value, indicating CoP excursions in the anteroposterior direction, and mediolateral trajectory value, showing the amount of CoP displacement in the mediolateral direction, were calculated using the Win-Track software (Medicapteurs Technology, Balma, France). Average CoP excursion velocities in the anteroposterior and mediolateral directions were also analyzed.

Muscle thickness measurements were obtained using ultrasound by a physical medicine and rehabilitation specialist with 10 years of experience in musculoskeletal ultrasound assessment. A Mindray DC-T6 (Mindray Bio-medical Electronics Co., Ltd., Shenzhen, China) ultrasound device with a 2-5 mHz curved linear probe was used for measurements. All muscle thickness measurements were obtained in duplicate for each muscle, and average values were included in the analyses. The thickness of the multifidus muscle was measured in the prone position. For this purpose, the probe was longitudinally placed over the lumbar spine and centered at the transverse process of the vertebra at the level measured. Afterward, the probe was advanced laterally and slightly angled medially until the facet joint at that level was visualized. The multifidus muscle thickness was determined by measuring the distance between the facet joint and the fascia (Figure 1a). Measurements were obtained from the lumbar region at four levels in the L1-2, L2-3, L3-4, and L4-5 facet joints. Multifidus muscle thickness measurements with ultrasound have been shown to produce highly reliable results, particularly when performed by a single examiner.[16]

The thickness of the gastrocnemius muscle (medial and lateral) was measured with the subject in the prone position and both feet hanging off the edge of the bed.[17] Measurements were obtained by longitudinally placing the transducer over the muscle at 30% proximally between the lateral condyle of the tibia and the lateral malleolus. The distance between the superficial and deep fascia was measured for each muscle (Figure 1b and 1c).[18]

Statistical analysis

A power analysis was conducted using the G*Power software version 3.1 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) to determine the sample size. Using the mean and standard deviation data on paraspinal muscle thickness from the literature[7] for patient and control groups, the effect size was calculated as 0.63. It was estimated that a sample size of 32 patients in each group would be required for the study, assuming alpha=0.05 and 1-beta=0.80.

Data were analyzed using IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis the study findings. Descriptive statistics were summarized as mean, median, standard deviation, and minimum-maximum. For between-group comparisons, Student's t-test was used for quantitative data with a normal distribution, and the Mann-Whitney U test was used for quantitative data without a normal distribution. Relationships between muscle thickness and other parameters were examined using Spearman's rank correlation analysis. The significance level was set at $p<0.05$. Intraclass correlation coefficients (ICC) were calculated to evaluate intrarater reliability of muscle thickness measurements by ultrasound.

RESULTS

No significant difference was found between the groups in terms of mean age and sex ($p=0.201$ and $p=0.784$, respectively). The mean body mass index of the AS group (26.3±4.2 kg/m^2) was similar to that of the control group (25.6±3.3 kg/m^2, $p=0.493$). Among AS patients, there were 28 patients on TNF-alpha inhibitors and four patients on nonsteroidal anti-inflammatory drugs. Ankylosing spondylitis patients had a median disease duration of 10 (range, 2 to 32) years, with a BASDAI score of 2.7 (0.2-7.4), a BASFI score of 1.7 (0-7.3), and a BASMI score of 3.2 (0.2-6.0).

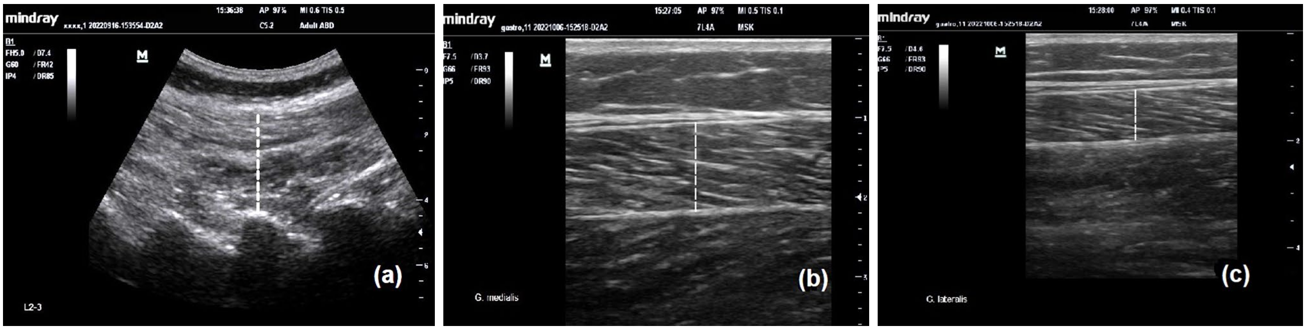

Figure 1. (a) Ultrasound measurement of the multifidus muscle thickness at the level of L2-L3 facet joint. **(b)** Ultrasound measurements of muscle thickness of medial gastrocnemius muscles. **(c)** Ultrasound measurements of muscle thickness of lateral gastrocnemius muscles.

G: Gastrocnemius; L: Lumbar.

TABLE 1
Comparison of CoP excursions between the groups in the standard stance

	AS group		Control group		
	Median	Min-Max	Median	Min-Max	*p*
EOCoPT-$_{\text{Trajectory}}$	52.1	28.1-162.3	49.4	30.4-77.9	0.382
EOCoPAP-$_{\text{Velocity}}$	1.3	0.6-3.4	1.0	0.6-1.8	0.176
EOCoPML-$_{\text{Velocity}}$	1.0	0.5-3.5	0.9	0.6-1.9	0.486
EOCoPAP-$_{\text{Trajectory}}$	0.9	0.4-3.0	0.9	0.2-2.6	0.614
EOCoPML-$_{\text{Trajectory}}$	0.8	0.2-1.9	0.6	0.1-2.1	0.190
ECCoPT-$_{\text{Trajectory}}$	82.3	45.2-190.5	72.8	37.8-103.9	0.038*
ECCoPAP-$_{\text{Velocity}}$	2.0	1.2-4.5	1.6	0.8-2.4	0.006**
ECCoPML-$_{\text{Velocity}}$	1.6	0.7-5.0	1.3	0.7-2.5	0.149
ECCoPAP-$_{\text{Trajectory}}$	1.9	0.6-4	1.1	0.6-3.1	0.003**
ECCoPML-$_{\text{Trajectory}}$	0.9	0.3-3.7	0.8	0.3-3.6	0.173

CoP: Center of pressure; AS: Ankylosing spondylitis; EO: Eyes open; EC: Eyes closed; AP: Anteroposterior; ML: Mediolateral; T: Total; * p<0.05; ** p<0.01.

In the patient group, the ICC values for the multifidus muscles at the L1-2, L2-3, L3-4, and L4-5 levels and for the medial and lateral gastrocnemius muscles were 0.979 (95% confidence interval [CI]: 0.952 to 0.991), 0.961 (95% CI: 0.914 to 0.983), 0.972 (95% CI: 0.937 to 0.988), 0.962 (95% CI: 0.915 to 0.983), 0.960 (95% CI: 0.910 to 0.983), and 0.974 (95% CI: 0.941 to 0.989), respectively. The ICC values for the same muscles in the control group were 0.982 (95% CI: 0.958 to 0.993), 0.976 (95% CI: 0.944 to 0.990), 0.981 (95% CI: 0.954 to 0.992), 0.964 (95% CI: 0.915 to 0.985), 0.963 (95% CI: 0.913 to 0.984), and 0.966 (95% CI: 0.919 to 0.986), respectively.

Compared to controls, no signs of postural instability were observed in AS patients during measurements of CoP excursion in the standard stance position with the eyes open (Table 1). On measurements in the same position with the eyes closed, AS patients showed a significantly greater median CoP excursion velocity and trajectory in the anteroposterior direction versus controls. The total CoP trajectory was also significantly increased in AS patients. No evidence of postural instability was observed in the mediolateral direction (Table 1).

However, on measurements with the eyes open in tandem stance, greater CoP excursions were found

TABLE 2
Comparison of CoP excursions between the groups in the tandem stance

	AS group		Control group		
	Median	Min-Max	Median	Min-Max	*p*
EOCoPT-$_{\text{Trajectory}}$	453.6	252.1-1560.7	350.1	49.3-509.7	0.006**
EOCoPAP-$_{\text{Velocity}}$	13.0	6.3-48.1	9.6	2.9-15.5	0.007**
EOCoPML-$_{\text{Velocity}}$	6.8	4.1-16.8	5.1	2.2-8.1	0.019*
EOCoPAP-$_{\text{Trajectory}}$	6.8	3.7-20.9	4.5	3.0-9.5	0.004**
EOCoPML-$_{\text{Trajectory}}$	3.6	1.9-9.0	2.9	1.2-4.6	0.024*
ECCoPT-$_{\text{Trajectory}}$	922.1	465.5-1588.6	621.4	294.8-854.8	0.001**
ECCoPAP-$_{\text{Velocity}}$	23.0	8.4-44.4	12.5	8.3-17.0	<0.001**
ECCoPML-$_{\text{Velocity}}$	15.3	1.2-29.0	9.0	4.2-16.7	<0.001**
ECCoPAP-$_{\text{Trajectory}}$	9.3	4.8-24.3	7.0	4.0-11.0	<0.001**
ECCoPML-$_{\text{Trajectory}}$	7.1	4.8-19.2	4.1	2.2-8.2	<0.001**

CoP: Center of pressure; AS: Ankylosing spondylitis; EO: Eyes open; EC: Eyes closed; AP: Anteroposterior; ML: Mediolateral; T: Total; * p<0.05; ** p<0.01.

TABLE 3
Comparison of CoP excursions between the groups in the single-leg stance

	AS group		Control group		
	Median	Min-Max	Median	Min-Max	*p*
$CoPT_{Trajectory}$	537.2	268.1-1331.9	383.9	242.7-587.1	0.010*
$CoPAP_{Velocity}$	12.3	5.6-25.2	9.0	5.6-16.6	0.001**
$CoPML_{Velocity}$	8.8	4.3-29.8	7.3	4.1-13.2	0.016*
$CoPAP_{Trajectory}$	4.5	2.3-11.0	3.2	1.4-4.9	0.003**
$CoPML_{Trajectory}$	3.6	1.5-31.9	3.0	1.2-4.9	0.033*

CoP: Center of pressure; AS: Ankylosing spondylitis; EC: Eyes closed; AP: Anteroposterior; ML: Mediolateral; T: Total; * p<0.05; ** p<0.01.

TABLE 4
Between-group comparison of muscle thickness as measured by ultrasound

	AS group	Control group	
	Mean±SD	Mean±SD	*p*
Multifidus muscle			
L_{1-2}	22.3±3.6	26.0±4.3	0.002**
L_{2-3}	24.4±3.4	29.3±4.7	<0.001**
L_{3-4}	27.2±4.4	31.6±4.2	0.001**
L_{4-5}	29.7±5.2	33.0±3.7	0.017*
Gastrocnemius muscle			
Medial	18.5±2.0	20.6±2.1	0.002**
Lateral	15.4±1.7	17.5±2.5	0.002**

SD: Standard deviation; L: Lumbar; * p<0.05; ** p<0.01.

in both mediolateral and anteroposterior directions in AS patients. Much greater CoP excursions were observed in both directions on measurements with the eyes closed in the tandem stance (Table 2).

Postural instability in both anteroposterior and mediolateral directions was evident in the AS group on measurements in single-leg stance on the dominant side (Table 3).

Comparing muscle thickness measurements between the groups, the AS group showed reduced thickness for all muscles, including the multifidus muscle at L1-2, L2-3, L3-4, and L4-5 segments and medial and lateral gastrocnemius muscles (Table 4).

TABLE 5
Relationships between muscle thickness and parameters of static and dynamic balance in AS patients

	SS-EO-CoPT	SS-EC-CoPT	Tandem-EO-CoPT	Tandem-EC-CoPT	Single leg-CoPT	TUG test
Multifidus muscle						
L_{1-2}						
Rho	-0.016	-0.409**	-0.288	-0.234	-0.225	-0.700**
p	0.915	0.005	0.055	0.177	0.137	<0.001
L_{2-3}						
Rho	-0.114	-0.392**	-0.237	-0.221	-0.183	-0.535**
p	0.445	0.007	0.113	0.195	0.225	0.007
L_{3-4}						
Rho	-0.022	-0.395**	-0.122	-0.222	-0.028	-0.666**
p	0.886	0.007	0.424	0.200	0.854	0.001
L_{4-5}						
Rho	-0.001	-0.343*	-0.079	-0.137	0.016	-0.613**
p	0.995	0.021	0.604	0.432	0.916	0.002
Gastrocnemius muscle						
Medial						
Rho	-0.086	-0.307*	-0.373*	-0.153	-0.387**	-0.611**
p	0.570	0.040	0.011	0.374	0.008	0.002
Lateral						
Rho	-0.115	-0.452**	-0.203	-0.224	-0.354*	-0.690**
p	0.445	0.002	0.176	0.189	0.016	<0.001

CoPT: centre of pressure total, EC: eye closed, EO: eye open, L: lumbar, Rho: coefficient of correlation, SS: standard stance, TUG test: Timed Up And Go test

A negative correlation was found between all muscle thickness values and TUG scores in AS patients (Table 5). Center of pressure excursions were not correlated with muscle thickness measurements in the standard stance with the eyes open. However, CoP excursion trajectory showed a negative correlation with all muscle thickness measurements with the eyes closed in the same stance. In the tandem position, a weak negative correlation was found between CoP excursions and only the medial gastrocnemius muscle thickness on measurements with the eyes open, whereas no correlation was observed between muscle thickness values and CoP excursions on measurements with the eyes closed. Center of pressure excursions showed a negative correlation with gastrocnemius muscle thickness (both medial and lateral) in the single-leg stance measurements from the dominant side (Table 5).

DISCUSSION

The results of our study showed that lumbar multifidus and gastrocnemius muscles were thinner in AS patients compared to the control group. Reduced muscle thickness was found to be associated with the impairment of static and dynamic balance. Increased prevalence of vertebral fractures in patients with AS is a well-known fact. Identification and correction of the factors associated with impaired balance that can cause falls and fractures are important for AS patients. Reduced physical performance has been associated with a higher number of falls in AS patients, particularly in those with a long disease duration and decreased functional capacity.[19] Murray et al.'s[1] paper was among the first reports highlighting balance problems in AS patients. They demonstrated balance impairment in these patients, which was worse when the eyes were closed.

In contrast with the literature, Aydoğ et al.[20] reported that AS does not have a negative effect on postural stability. However, it is noteworthy that all balance tests were conducted with the eyes open in that study. Similarly, in our study, no postural instability was observed in AS patients during CoP excursion tests performed in the standard stance with the eyes open. In addition, there was no correlation between muscle thickness of multifidus and gastrocnemius muscles and the magnitude of CoP sway when measured with the eyes open. Static balance is not impaired in AS patients due to compensatory mechanisms when the eyes are open. It is possible that, due to this compensatory effect resulting from receiving visual input when the eyes are open, the magnitude of CoP excursions was not correlated with muscle thickness in this condition. The finding of postural instability in AS patients in the anteroposterior direction on CoP sway tests in the standard stance with the eyes closed in our study is consistent with previous reports. Batur and Karataş[6] showed a significant increase in postural sway in the anteroposterior direction in AS patients compared to controls. In the same study, mediolateral sway was similar in AS patients and controls. Gündüz et al.[21] demonstrated impairment of both static and dynamic balance in AS patients, with static balance impaired in the eyes-closed condition. Vergara et al.[2] reported a significantly greater CoP displacement in the AS group when tested on a 'force platform' with the eyes closed. No such changes were observed in the control group in that study.

Our findings showed that postural instability occurred in both anteroposterior and mediolateral directions during the tandem stance, even when the eyes were open. Postural instability is further increased when the eyes are closed in the tandem stance. In a study by Çınar et al.[22] examining balance problems in AS patients using clinical scales, altered static balance versus controls was found only in the tandem stance with the eyes closed when assessed by the Romberg test. In the current study, significant increases in CoP excursions in both anteroposterior and mediolateral directions were also observed in the single-leg stance with the eyes open in AS patients. Our findings from the TUG test indicate that dynamic balance is impaired in patients with AS. According to Çınar et al.,[22] all of the tests that are used to assess dynamic balance, such as the Berg Balance Scale, ABC scale, and five times Sit-to-Stand test, show pathological findings in AS patients. Additionally, Durmuş et al.[5] demonstrated problems with dynamic balance in these patients using the Balance System.

In the present study, reduced muscle thickness was observed in the multifidus muscle at all lumbar segments (L1-2, L2-3, L3-4, and L4-5) as well as the medial and lateral gastrocnemius muscles in AS patients compared to the control group. These findings are in line with those reported by former studies, which are few in number. Resorlu et al.[7] showed a reduction in the cross-sectional areas of the paravertebral muscles at all lumbar segments (L1-2, L2-3, L3-4, and L4-5) in AS patients compared to control subjects. In a recently published study, denervation of the paraspinal muscles was noted in AS patients.[8] Resorlu et al.[7] suggested that paraspinal muscle atrophy in AS patients possibly results from a number of factors, including immobilization due to pain and increased cytokine levels. Weakness of some core muscles, such as back extensors, was reported in AS patients compared to controls. Paraspinal muscle atrophy has been implicated in reduced muscle strength.[3] In a another study, a correlation was reported between balance and exercise endurance times of core muscles, which are crucial for maintaining postural stability, in AS patients.[9]

The negative correlation between the TUG results and muscle thickness of the multifidus and gastrocnemius muscles, as found in our study, demonstrates that reduced muscle thickness adversely affects dynamic balance. Our findings show that as the multifidus and gastrocnemius muscles get thinner, static balance is also impaired in the standard stance with the eyes closed. In the tandem stance, increased postural instability was correlated with reduced thickness of the medial gastrocnemius muscle. On single stance balance tests, reduced thickness of the medial and lateral gastrocnemius muscles caused postural instability in AS patients. Collectively, these findings show that a significant loss occurs in the mentioned muscles in patients with AS, and this muscle loss has negative effects on both static and dynamic balance. The demonstration of the association of muscle thickness as measured by ultrasound with balance in AS patients is important. In these patients, early detection of muscle loss seems possible using ultrasound, a relatively inexpensive and easy-to-use tool. This will potentially contribute to early rehabilitation of affected muscles with the help of appropriate exercise programs and prevention of falls. It is known that muscle tissue has an important role in providing proprioceptive information in addition to maintaining postural stability through contraction. Balance exercises with the eyes closed were reported to be particularly effective in improving balance in AS patients, and this improvement was attributed to favorable effects of rehabilitation on proprioception.[23]

It has been shown that in addition to balance problems, the gait cycle is also adversely affected in patients with AS. Gait pathologies, including reduced plantar flexion in the heel strike of the gait cycle, were detected in those patients.[24] As substantial alterations in the sagittal plane have been reported, the relationship between muscle problems and such pathological changes in the gait merits further investigation.

Our study is valuable since this is the first study to demonstrate the association between muscle thickness of the multifidus and gastrocnemius muscles and balance in patients with AS. However, some limitations should be noted for our study. A relatively low number of patients were included in the study, and the frequency of falls and fractures were not assessed with long-term follow-up. Furthermore, most of the patients in our patient group were receiving treatment with biologics.

In conclusion, patients with AS have significantly thinner lumbar multifidus and gastrocnemius muscles compared to the healthy population. Reduced thickness of these muscles negatively affects static and dynamic balance. Musculoskeletal ultrasound can be used to detect reductions in muscle thickness. Undoubtedly, postural instability does not pose a clinical concern for all AS patients. Nevertheless, it is possible to prevent falls and fractures through early implementation of appropriate rehabilitation programs, particularly in patients with risk factors for fractures, following an assessment of balance when deemed necessary and taking into account muscle loss.

Ethics Committee Approval: The study protocol was approved by the Istanbul Medeniyet University Göztepe Training and Research Hospital Ethics Committee (date: 23.03.2017, no: 2017/0003). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Concept, design, data collection,analysis,literature research, writing, review: E.M.; Design, supervision, materials, data collection, literature research, writing, review: N.M.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

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