

# **Respiratory muscle strength and pulmonary functions in athletes:** differences by BMI classifications

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#### Abstract

**Background and objective**: The respiratory capacity, which substantially affects exercise performance, tends to be affected by many factors such as anthropometric characteristics and different sports branches. We know which body mass index (BMI) category negatively affects pulmonary functions (PFs) in sedentary, but it is unclear in the athlete population. Thus, the first aim of this study was to compare respiratory muscle strength (RMS) and PFs in athletes according to BMI categories. Furthermore, we examined whether different sports disciplines affect RMS and PFs as a second aim in the study. **Methods**: Athletes were divided into four groups according to BMI categories (<18.5, 18.5–24.9, 25.0–29.9, and  $\geq 30.0 \text{ kg/m}^2$ ) and two groups (individual and team) according to their sport disciplines. **Results**: The results showed that significant differences in MIP (cmH<sub>2</sub>O), MEP (cmH<sub>2</sub>O), FVC (lt), and FEV<sub>1</sub> (lt) scores according to BMI categories (p < 0.001 and p < 0.05). We found that the highest RMS scores were in the 18.5–24.9 and 25.0–29.9 kg/m<sup>2</sup> BMI categories (p < 0.001 and p < 0.05). Also, it was revealed that individual athletes' MIP, MEP, FVC, and FEV<sub>1</sub> scores were higher than others in sports disciplines (p < 0.001 and p < 0.05). **Conclusion**: These findings suggest that athletes' best RMS and PFs scores can be obtained in the 18.5–24.9 or 25.0–29.9 kg/m<sup>2</sup> BMI categories. Accordingly, we consider that different BMI values have varied effects on the athletes' respiratory capacities and should be kept under constant control. Also, individual athletes had the highest RMS and PFs due to the characteristics of sports disciplines.

Keywords: Body mass index; Respiratory functions; Sports disciplines; Individual athletes; Team athletes

## 1. Introduction

Respiratory functions are one of the most critical factors affecting exercise performance, and its functioning is mechanically dependent on the capacity of respiratory muscles [1]. It is generally accepted that elite athletes and physically active individuals tend to have the higher respiratory capacity, and their respiratory capacity is affected by many factors such as metabolism, physiology (strength, agility, power, speed, cardiovascular endurance, etc.), and anthropometry [2–4].

Firstly, it is common to monitor body composition in sports associated with performance. Generally, indexes calculated by superficial anthropometries, such as body mass index (BMI), are often preferred for convenience and practicality [5]. BMI, a significant anthropometric indicator, is between the essential variables used for predicting metabolic and cardiovascular disease risks [6]. Specifically, a simple index is calculated with the participant's mass and height, which is used to classify individuals into underweight, normal, overweight, and obese in medical and sports medicine research [7,8]. Actually, BMI singly does not provide precise, valid estimates of underweight, obesity, or other classifications in elite athletes, but it can give some opinion.

A high BMI can negatively affect physical performance. Conversely, underweight is equally a concern for

the performance of athletes. It is known that excessive body weight decreases aerobic capacity and exercise tolerance as excessive weight adversely affects the functions of the diaphragm and thoracic structures [7,9]. Also, such mechanical factors cause a reduction in airway caliber, increased airway resistance, and a significant decrease in lung volumes [10–14]. Several studies in sedentary people showed that respiratory capacities vary according to different BMI categories [15–17]. Therefore, BMI can significantly affect respiratory capacities in athletes as well as in sedentary [18,19].

Secondly, individual and team sports are challenging activities. When athletes competing in these disciplines are challenged to reach a high level, this can lead to physiological adaptations such as endurance, speed, power, or strength. Therefore, physiological features such as muscle, cardiovascular and respiratory systems, which increase with these adaptations in athletes, benefit their athletic performances [3,20]. Especially athletes need vigorous or high-intensity sessions to maintain and facilitate high physical condition forms. Accordingly, it is known that the respiratory system plays active roles during regularly performed high-intensity exercises, and it is exposed to adaptive changes over time according to the exercise type [21,22]. Previous studies have suggested that respiratory capacity may differ according to some branches because

Copyright: © 2022 The Author(s). Published by IMR Press. This is an open access article under the CC BY 4.0 license. sports branches differ in exercise type, intensity, duration, and frequency [3,4,20,23]. Specifically, such factors partially adapt the respiratory system and result in greater lung volumes and capacities in elite athletes [3,4].

This study aimed to compare respiratory muscle strength (RMS) and pulmonary functions (PFs) in athletes according to BMI categories, and the second aim was to examine whether different sports disciplines affect RMS and PFs.

# 2. Materials and methods

# 2.1 Study design

This study was designed as a cross-sectional study. All measurements were made at Yaşar Doğu Sport Science Faculty Performance Laboratory during the 2019– 2020 academic year. Participants visited the laboratory two times, and all measurements were completed in three weeks. During the first visit, they were informed about the test protocols, and a pilot application was performed to help them understand how the study would progress. We also measured the physical characteristics of the participants during this visit. All measurement trials were conducted at the same time of day ( $\pm 1$  h) to minimize the effect of diurnal variation. Participants were required to consume their last meal at least 3 h before and refrain from drinking caffeinated beverages at least 10 h before the test.

#### 2.2 Participants

The study population consisted of 271 participants included in three different groups, namely the individual athletes (97), team athletes (134), and sedentary (40). We classified the athletes into two subgroups: individual athletes (athletics, cycling, rowing, martial arts) and team athletes (basketball, soccer, volleyball). Athlete groups were composed of those trained regularly for at least three years for 6 hours a week. All of them were non-smokers and do not have any respiratory disorders. The study was approved by the Clinical Research Ethics Committee University of Ondokuz Mayıs (Ruling No: 2020/158) and conducted following the Declaration of Helsinki. Each participant provided written informed consent.

#### 2.3 Measurements

# 2.3.1 Anthropometry

Anthropometric measurements such as height (with sports clothes without shoes) and weight (anatomical position) were assessed (precision to 0.1 cm and 0.01 kg, respectively) (SECA, Hamburg, Germany). The stadiometer and scale were calibrated periodically during the study. BMI was calculated as the ratio of mass (kilograms) divided by height (meters) squared (kg/m<sup>2</sup>). We used the World Health Organization (WHO) ranges for BMI categories. Accordingly, we categorized the athletes' BMI in 4 groups as <18.5, 18.5–24.9, 25.0–29.9 and  $\geq$ 30.0 kg/m<sup>2</sup> [24].

#### 2.3.2 Pulmonary function test

For the determination of lung volumes, Spirometry (CPFS/D USB Spirometer, MGC Diagnostics, Saint Paul, MN, USA) was used, and pulmonary function test followed the American Thoracic Society and the European Respiratory Society (ATS/ERS) guidelines [25]. Participants underwent the test in a sitting position, wearing a nose clip. After a maximum inhalation, participants were asked to seal their lips entirely around the mouthpiece and exhale for 6 s as hard and fast as possible. Pulmonary function tests were performed three times for each participant, and the best value was recorded. The forced vital capacity (FVC) (liter), forced expiratory volume for one second (FEV<sub>1</sub>) (liter), and Tiffeneau-Pinelli index (FEV<sub>1</sub>/FVC) (%) were obtained by direct measurements. The maximum voluntary ventilation (MVV) (lt/min) was also calculated over 12 s.

#### 2.3.3 Respiratory muscle strength

Maximal inspiratory (MIP) and expiratory pressures (MEP) were measured with a portable manometer having a flanged mouthpiece (MicroRPM, CareFusion Micro Medical, Kent, UK) and were assessed according to the published guidelines [26]. All measurements were made with seated position looking straight ahead and wearing a nose clip. We asked each participant to exhale to residual volume and then to inhale to total lung capacity (pressure sustained for at least 1.5 s) when measuring maximal inspiratory and expiratory pressures, respectively. The maneuver was repeated five times, and the mean of three acceptable trials (coefficient of variation less than 5%) was recorded as MIP and MEP scores.

### 2.4 Statistical analysis

The statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA), and all figures were illustrated with GraphPad Prism 8.4.3 program (GraphPad Software Inc., San Diego, CA, USA). Data were expressed as the median, minimum, maximum, and interquartile range (IQR). We analyzed the normality assumption with the Kolmogorov-Smirnov test. The differences in values between groups were assessed using the Kruskal-Wallis H test. The statistical *p* values < 0.05 were considered to indicate significance. Spearman's rank correlation was performed to determine the relation between respiratory muscle strength and pulmonary functions. The sample size was justified by a priori power analysis in G\*power using a targeted alpha ( $\alpha$ ) = 0.05, power (1- $\beta$ ) = 0.95, effect size (*f*) = 0.241, and found as 270 [4].

## 3. Results

All participants' age, height, weight, and BMI data according to gender and sports branches were presented in Tables 1,2.

		Age (year)	Height (cm)	Weight (kg)	BMI (kg/m <sup>2</sup> )
Individual athletes (n = 97)	Female	18 (18–25)	176 (165–179)	65 (61–72)	21 (20–24)
	Male	20 (19–23)	178 (173–181)	71 (68–77)	23 (22–24)
	Total	20 (18–23)	178 (173–180)	71 (66–76)	23 (22–24)
Team athletes $(n = 134)$	Female	23 (19–26)	175 (170–181)	66 (57–71)	21 (20–23)
	Male	19 (18–23)	179 (173–183)	71 (65–80)	22 (21–25)
	Total	21 (18–24)	177 (172–183)	70 (63–75)	22 (20–24)
Sedentary (n = 40)	Female	26 (22–34)	165 (161–180)	59 (55–73)	21 (21–22)
	Male	29 (27–31)	177 (170–180)	79 (70–89)	25 (24–27)
	Total	28 (26-32)	173 (165–180)	72 (59–86)	23 (21–27)

Table 1. Participants' physical characteristics according to sports disciplines (Median-IQR) (n = 271).

IQR, interquartile range; BMI, Body mass index.

Table 2. Participants' physical characteristics according to sport branches (Median-IQR) (n = 271).

		Age (year)	Height (cm)	Weight (kg)	$BMI (kg/m^2)$
Individual	Athletics (35)	19 (19–20)	179 (177–182)	70 (68–72)	22 (21–23)
	Cycling (16)	22 (20–26)	179 (173–183)	75 (70-83)	24 (22–24)
	Rowing (11)	17 (17–18)	172 (170–179)	64 (56–77)	22 (19–24)
	Martial arts (35)	22 (18–24)	176 (170–180)	74 (66–81)	24 (22–26)
	Iath Total (97)	20 (18–23) <sup>b</sup>	178 (173–180) <sup><i>a</i>,<i>b</i></sup>	71 (66–76)	23 (22–24) <sup>a</sup>
Team	Basketball (49)	23 (18–29)	181 (176–185)	71 (64–80)	22 (20–24)
	Soccer (56)	19 (18–22)	174 (170–180)	69 (63–75)	22 (21–24)
	Volleyball (29)	23 (21–24)	180 (171–183)	68 (58–73)	21 (20–23)
	T <sub>ath</sub> Total (134)	21 (18–24) <sup>b</sup>	177 (172–183) <sup>b</sup>	70 (63–75)	22 (20–24) <sup>b</sup>
	S <sub>ed</sub> Total (40)	28 (26–32) <sup>a</sup>	173 (165–180) <sup>a</sup>	72 (59–86)	23 (21–27) <sup>a</sup>
	<i>p</i> -values	< 0.001	0.034	0.197	0.009

<sup>a,b</sup>No significant differences between groups that share the same letter in the same column. I<sub>ath</sub>,

Individual athletes;  $T_{ath}$ , Team athletes;  $S_{ed}$ , Sedentary; IQR, interquartile range; BMI, Body mass index.

Significant differences were observed in RMS and PFs according to BMI categories. What is remarkable about this graph was that the highest MIP [122 (99–156); 110–148], MEP [153 (115–187); 136–170], FVC [5.72 (4.33–6.43); 4.82–6.08], and FEV<sub>1</sub> [4.69 (3.76–5.55); 4.22–5.12] scores were found in athletes in the BMI category 25.0–29.9 kg/m<sup>2</sup>. Also, athletes in <18.5 kg/m<sup>2</sup> BMI category had the lowest RMS and PFs (p < 0.001, p < 0.05) (Fig. 1).

There were significant differences among the three groups in terms of RMS and PFs. The results showed that  $I_{ath}$  had greater MIP [119 (84–156); 107–130], MEP [143 (87–187); 123–166], FVC [5.14 (3.55–6.15); 4.75–5.61] and FEV<sub>1</sub> [4.59 (3.11–5.73); 4.13–4.97] scores when compared to  $T_{ath}$  and  $S_{ed}$  (p < 0.001, p < 0.05) (Fig. 2). No significant difference between the three groups was evident in MVV.

As shown in the graph, the results show that the FVC [5.27 (4.11–6.15); 4.98–5.63]; [5.33 (3.90–6.43); 4.82–5.83] and FEV<sub>1</sub> [4.68 (3.65–5.73); 4.28–5.00]; [4.70 (3.10–

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5.80); 4.31–5.11] scores of male athletes in both sports disciplines (I<sub>ath</sub> and T<sub>ath</sub>, respectively) are higher than S<sub>ed</sub> male (p < 0.001). However, there was no significant difference between females in RMS and PFs, respectively, medians (min–max) with IQR (Fig. 3).

Fig. 4 shows that the correlation between RMS and PFs. Whereas MIP scores in individual athletes showed a significant positive correlation with MVV ( $\mathbf{r} = 0.271$ , p = 0.001), no correlation was seen at FVC and FEV<sub>1</sub>. MEP had a significant correlation with FVC ( $\mathbf{r} = 0.297$ ), FEV<sub>1</sub> ( $\mathbf{r} = 0.288$ ) and MVV ( $\mathbf{r} = 0.275$ ) (p = 0.001 for all). Team athletes' MIP and MEP significantly correlated with FVC ( $\mathbf{r} = 0.292$ ;  $\mathbf{r} = 0.355$ ), FEV<sub>1</sub> ( $\mathbf{r} = 0.330$ ;  $\mathbf{r} = 0.366$ ) ve MVV ( $\mathbf{r} = 0.409$ ;  $\mathbf{r} = 0.430$ ), respectively (p = 0.001 for all). It was seen that sedentary MIP scores are correlated with FEV<sub>1</sub> ( $\mathbf{r} = 0.339$ , p = 0.05) and MVV ( $\mathbf{r} = 0.324$ , p = 0.05).



Fig. 1. Respiratory muscle strength and pulmonary functions according to BMI categories in athletes [<18.5<sub>(n=10)</sub>; 18.5–24.9<sub>(n=190)</sub>; 25.0–29.9<sub>(n=23)</sub>;  $\geq$ 30.0<sub>(n=8)</sub>]. All data were expressed as median (min–max). (A) Respiratory muscle strength. (B,C) Pulmonary functions (n = 231). <sup>*a,b*</sup>No significant differences between groups that share the same letter. \*\*p < 0.001; \*p < 0.05; ns, non-significant; MIP, Maximal inspiratory pressure; MEP, Maximal expiratory pressure; FVC, Forced vital capacity; FEV<sub>1</sub>, Forced vital capacity at 1 second; MVV, Maximal voluntary ventilation.



Fig. 2. Respiratory muscle strength and pulmonary functions between groups  $[I_{ath(n=97)}; T_{ath(n=134)}; S_{ed(n=40)}]$ . All data were expressed as median (min-max). (A) Respiratory muscle strength. (B,C) Pulmonary functions (n = 271). <sup>*a,b,c*</sup>No significant differences between groups that share the same letter. \*\*p < 0.001; \*p < 0.05; ns, non-significant;  $I_{ath}$ , Individual athletes;  $T_{ath}$ , Team athletes;  $S_{ed}$ , Sedentary; MIP, Maximal inspiratory pressure; MEP, Maximal expiratory pressure; FVC, Forced vital capacity; FEV<sub>1</sub>, Forced vital capacity at 1 second; MVV, Maximal voluntary ventilation.

## 4. Discussion

This study demonstrates that the athletes' respiratory muscle strength and pulmonary functions according to the BMI categorization. The other aim was to compare the respiratory capacities according to sports disciplines. Also, we examined the relationship between respiratory muscle strength and pulmonary functions in sports disciplines. For these purposes, we obtained four significant results: (1) Respiratory muscle strength of athletes in the BMI categories 18.5–24.9 and 25.0–29.9 kg/m<sup>2</sup> was higher than the others (<18.5 and  $\geq$ 30.0 kg/m<sup>2</sup>), (2) athletes in the 25.0– 29.9 kg/m<sup>2</sup> category had the highest pulmonary functions, (3) RMS and PFs of athletes in individual disciplines were higher than athletes in other disciplines, and (4) mainly the MEP affected all PFs (FVC, FEV<sub>1</sub>, MVV). At present, many researchers have examined the relationship between different BMI categories and pulmonary functions at sedentary and stated that this index is among the most important factors affecting respiratory capacities [9,14]. This study showed that athletes in the 25.0–29.9 kg/m<sup>2</sup> BMI category had higher MIP, MEP, FVC, and FEV<sub>1</sub> scores than athletes in other categories. According to BMI categories in the athlete population, there is no study investigating respiratory functions, but we have seen that athletes with different BMI values are compared in some studies. Hackett *et al.* [27] showed that athletes with a BMI of 29.3  $\pm$  2.3 had higher MIP and MEP (199–267) scores than those with 22.5  $\pm$  1.8 (139–175). Several studies have reported that individuals in the BMI category of 18.5–24.9 kg/m<sup>2</sup> (ideal BMI) have higher respiratory ca-



Fig. 3. Respiratory muscle strength and pulmonary functions of male  $[I_{ath(n=82)}; T_{ath(n=84)}; S_{ed(n=20)}]$  and female  $[I_{ath(n=15)}; T_{ath(n=50)}; S_{ed(n=20)}]$  between groups. Data were expressed as median (min-max). (A) Respiratory muscle strength. (B,C) Pulmonary functions. <sup>*a,b*</sup>No significant differences between groups that share the same letter. \*\*p < 0.001; ns, non-significant;  $I_{ath}$ , Individual athletes;  $T_{ath}$ , Team athletes;  $S_{ed}$ , Sedentary; MIP, Maximal inspiratory pressure; MEP, Maximal expiratory pressure; FVC, Forced vital capacity; FEV<sub>1</sub>, Forced vital capacity at 1 second; MVV, Maximal voluntary ventilation.

pacities than individuals in the  $\geq$ 30.0 and <18.5 kg/m<sup>2</sup> categories [15–17]. It is known that excess body weight limits respiratory functions via various mechanisms, including the mechanical factors regarding the diaphragm, thoracic wall, abdomen, and upper airway, and this happens because of the prevention of complete thoracic expansion through restriction of thoracic wall movement by accumulating adipose tissue over the thoracic cage and abdomen [14,15].

Generally, excess body weight reduces chest wall function, creates greater metabolic demand for respiratory muscle contraction, and increases respiratory work-load [28]. Previous studies have indicated that respiratory muscle weakness is associated with skeletal muscle mass, resulting in decreased respiratory capacity [17,29]. Thus, the diaphragm, intercostal, and abdominal muscles that are respiratory muscles, play a role in maintaining PFs. When our study results were considered, it can be suggested that <18.5 and  $\geq$ 30.0 kg/m<sup>2</sup> BMI categories are a significant variable that affects the respiratory functions in athletes, as seen in sedentary people.

According to the present study results, the highest MIP, MEP, FVC, and FEV1 scores were seen in athletes in individual disciplines. In contrast, no significant difference was present between the groups regarding the MVV score. A study of various sports (swimming, wrestling, archery, basketball, and soccer) and inactive children (8-12 years) showed that athletes in individual disciplines had higher RMS than others [30]. Also, previous studies have shown that the RMS of swimmers, world-class powerlifters, football players, netball, and rugby players were higher when compared to sedentary [31-33]. On the other hand, several studies suggested that athletes of individual disciplines have higher PFs than the team and sedentary [30,34]. However, Durmic et al. [22] reported that athletes in team disciplines (football) are higher than individual disciplines (weightlifting, bodybuilding, defense sports) and sedentary. They explained that exercise activity leads to adaptive changes in spirometric parameters depending on the type of sports.

On the contrary, Lazovic et al. [35] reported no difference between sports disciplines. Although the literature has presented several inconsistent results, considering these studies, it is observed that compared branches have not been sufficiently diversified. Thus, this situation might have affected the study results differently. Our study showed that athletes competing in individual disciplines have stronger respiratory muscles and respiratory functions. These results support the claims that individual and team disciplines might improve respiratory capacities at different levels because they demand strength and endurance at different rates [4,30,34]. The respiratory capacities may manifest development at different levels, as athletes in different sports disciplines are characterized by different aerobic/anaerobic metabolism, the season for competition, training, and anthropometry. In this context, particularly the athletes from individual branches can exhibit their potentials/performances maximally during competitions, and training continuously exposes them to situations necessitating high ventilation. This situation might explain why the respiratory functions of individual athletes undergoing adaptation develop at different rates.

As shown in Fig. 3, MIP and MEP scores were not significant in males regarding sports disciplines. Male athletes in individual and team disciplines had higher FVC and  $FEV_1$  scores than sedentary males. No difference was present between the groups regarding the respiratory parameters of the female participants.

The study results revealed that RMS (MIP and MEP) affected the PFs (MVV, FVC, FEV<sub>1</sub>) performed with forced or temporal maneuvers. Similarly, MIP and MEP scores of athletes in various branches (cycling, running, rowing, swimming, rugby, netball, and underwater hockey) were determined to have relationships with FVC (r = 0.46;



Fig. 4. The correlation between respiratory muscle strength and pulmonary functions according to sports disciplines. \*p < 0.05; \*\*p < 0.001; ns, non-significant; RMS, Respiratory muscle strength; MIP, Maximal inspiratory pressure; MEP, Maximal expiratory pressure; FVC, Forced vital capacity; FEV<sub>1</sub>, Forced vital capacity at 1 second; MVV, Maximal voluntary ventilation.

0.41), FEV<sub>1</sub> (r = 0.42; 0.34), and MVV (r = 0.44; 0.37) [18]. Veteran athletes (aged >35 years) participating in world championships were determined to have a positive relationship only between the MEP and  $FEV_1$  scores [36]. On the contrary, Carten reported no relationship between RMS and PFs of athletes (swimmers, rugby, netball players) [31]. When the roles of cardiovascular and respiratory systems in oxygen transport are considered, strong respiratory muscles that help the lungs expand and diminish in size maximally are crucial for elite athletes [1,37]. During exercise, muscle strength must increase the lungs' volume with inspiration or return them to the expected size with expiration [38]. Even though expiration is a passive process, this situation becomes an active process activating the intercostal and abdominal muscles during vigorous exercise. In other words, it becomes a more active process to shorten

the expiratory time and to reduce the work-cycle at every breath as the demand for inspiration increases during exercise [39]. Thus, especially MEP can be considered to be an essential variable that affects almost all PFs (FVC, FEV<sub>1</sub>, MVV).

## 5. Conclusions

This study revealed that respiratory muscle strength and pulmonary functions differ according to BMI categories, and the athletes in the 18.5-24.9 and 25.0-29.9kg/m<sup>2</sup> BMI groups had the best respiratory parameters. The results showed that BMI values are effective on respiratory capacities and should be kept under control consistently in athletes. Secondly, we found that athletes in individual branches had higher respiratory capacity than others. Accordingly, it can be considered that sports disciplines that differ in many aspects improve the athletes' respiratory muscle strength and pulmonary functions at different levels.

# 6. Study limitations

Some limitations should be acknowledged regarding the present study. The major limitation was the inability to reach equal numbers of participants in categorized BMI groups despite researching a relatively large athlete population. The reason was that the population in the study consisted predominantly of athletes with ideal BMI values. Many studies have investigated the prevalence of BMI categories in athletes [40,41]. These studies may show us how difficult it is to reach the  $\geq$ 30.0 kg/m<sup>2</sup> BMI population in athletes. Thus, the results might not fully represent larger populations at different BMI categories. Still, the study data might enable or contribute to understanding the relationship between athletes' respiratory functions and BMI categories despite such a limitation.

## Abbreviations

BMI, Body mass index; RMS, respiratory muscle strength; PFs, pulmonary functions;  $S_{ed}$ , sedentary;  $I_{ath}$ , individual athletes;  $T_{ath}$ , team athletes; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume at 1 s; MVV, maximum voluntary ventilation over 12 s; MIP, maximal inspiratory; MEP, maximal expiratory pressures.

# Author contributions

EK: Conceptualization, methodology, writing original draft, visualization; ÖB: methodology, investigation, writing—review&editing; LB: review&editing.

#### Ethics approval and consent to participate

The study was approved by the Clinical Research Ethics Committee University of Ondokuz Mayıs (Ruling no: 2020/158), conducted in accordance with the World Medical Association Declaration of Helsinki. Each subject provided written informed consent.

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# **Conflict of interest**

The authors declare no conflict of interest.

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