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Investigating the Reliability of Ultrasonography Measurements of Masticatory Muscles in subjects with and without Temporomandibular Disorders: An Intra-Rater Reliability Study

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ABSTRACT

Introduction: Ultrasonography is a common tool for assessing muscle morphology. This study aims to investigate the intra-rater reliability of ultrasonography measurements of the masseter and lateral pterygoid muscles (LPM) in subjects with and without temporomandibular disorder (TMD) and to compare the measurements between the two groups.

Materials and Methods: A total of 30 individuals (15 subjects with TMD and 15 subjects without TMD) participated in the study. Imaging of the masseter muscle at rest and contraction, in addition to LPM, was performed using sonography in 2 sessions with a 2-day interval and the thickness of these muscles was measured. To assess the reliability, the intraclass correlation coefficient ($ICC_{(3, 3)}$), standard error of measurement, minimal detectable change, and limits of agreement were used. Additionally, for the between-group comparison of measurements, the independent sample t-test was used. The Cohen d was used to determine the size of the differences.

Results: $ICC_{(3, 3)}$ for intra-rater reliability of masseter thickness in rest and clenched positions and LPM were 0.89, 0.80, and 0.86 in the asymptomatic group, and 0.88, 0.80, and 0.90 in the TMD group, respectively. The values of absolute reliability parameters, such as standard error of measurement and minimal detectable change were in a small range. Additionally, compared to the asymptomatic group, the TMD group had thicker masseter muscle in the rest position and the effect size revealed a large difference between the groups. No significant differences were found between the groups for the thickness of the masseter muscle in contraction position and LPM.

Conclusion: The results of the intra-reliability study revealed that ultrasonography is a reliable method to measure the thickness of the masticatory muscles in TMD subjects and asymptomatic subjects. Additionally, TMDs can change the thickness of the masticatory muscles.

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1. Introduction

Temporomandibular disorders (TMD) are one of the main causes of orofacial pain [1]. The prevalence of TMDs is estimated at approximately 25% in the general population [2]. They are more common among women compared to men [2, 3]. Although the exact pathophysiology of TMDs is still unknown in many cases, evidence suggests that some specific pathology, such as congenital or developmental disorders, degenerative joint disorders, trauma, arthritis, systematic infection, and different conditions, including losing teeth, bruxism, and myofascial syndromes are associated with developing TMDs [4]. The classic clinical manifestations of TMDs include pain during normal living activities, such as eating, chewing, brushing, reduced jaw movements, and joint sounds. They can also be associated with other complications, such as headache, neck pain, tinnitus, and visual disorders [4-7].

In addition to clinical manifestations, some evidence suggested that the thickness, morphology, and activity pattern of muscles of mastication are affected by TMDs [8-12]. Masseter and lateral pterygoid muscles are two important muscles of the masticatory system [13]. The masseter muscle is a primary elevator of the mandible and participates in the mandibular protrusion [13]. Compared to the masseter the muscle, lateral pterygoid muscle (LPM) is deeply located and plays an active role during the mandibular depression (month opening), mandibular elevation (eccentrically), mandibular protrusion, and mandibular deviation [13].

In the early phases of TMDs, due to overuse, hypertrophy in masticatory muscles may be observed, and in chronic phases, the constant presence of pain may lead to disuse atrophy of the muscles [14]. Some studies have indicated that, compared to the asymptomatic subjects, TMD patients have a thicker masseter muscle [8-11]. Pekince et al. found [15] spasm points in the masseter muscle of the TMD subjects using sonography. Additionally, Ippolito et al. [16] found an increase in LPM thickness in TMD patients with articular disk displacement. Studies have also reported changes in the activity level of the LPM following pain and disability in TMD subjects.

There are several imaging techniques to assess the morphology and behavior of masticatory muscles and related soft tissues. Ultrasonography is a non-invasive valid tool that is widely used in the clinical setting to measure and quantify muscle thickness and cross-section [17-19]. Although several studies have used ultrasonography to measure the thickness of the masticatory muscles, little is known about the reliability of these measurements in TMD patients and asymptomatic subjects. Few studies have investigated the reliability of sonography measurements of masticatory muscles in asymptomatic subjects [14, 20] and despite the importance of reliability of measurements, to the best of our knowledge, there have been no studies that evaluate the reliability of sonography measurements of LPM muscles in TMD patients. Only one study [21] has assessed the reliability of sonography measurements of masseter muscle in TMD patients.

Accordingly, the first purpose of this study is to determine the intra-rater reliability of thickness of the masseter and LPM muscles in TMD patients and asymptomatic subjects, and the second purpose is to compare the measurements between the two groups.

2. Materials and Methods

A total of 15 TMD patients and 15 asymptomatic subjects in the age range of 20 and 45 were enrolled in this test re-test reliability study. Asymptomatic subjects were recruited from students and staff of the university while TMD patients were selected from patients of the Dental Clinic of Shariati Hospital and the Physiotherapy Clinic of the Physiotherapy Department of the University. The TMD subjects were referred by dentists and ear, nose, and throat specialists.

The diagnosis of TMDs was based on the diagnostic criteria for TMD (DC/TMD criteria). All TMD patients participating in this study had the following symptoms [7, 22]: Pain in the temporomandibular joint (TMJ) for more than 6 months; pain during the palpation of the masticatory muscles especially in the masseter muscle; and pain during jaw movements, such as eating and mouth opening. Some TMD patients reported headaches in the temporal area. In addition to the clinical examination, the TMD patients were asked to fulfill the TMD disability index questionnaire. This is a valid and reliable questionnaire that is used to evaluate the disability level of patients with TMDs. This tool has 10 items and assesses specific and non-specific functions of TMJ. Each item is scored from 0 (no disability) to 4 (severe disability) [23]. The maximum score for this questionnaire is 40. Raw scores are then transformed into a 0 to 100 scale [23]. If participants had a bilateral disorder, the side with more disability was selected for further evaluation.

Asymptomatic participants also were evaluated and reported no complaints of any pain or dysfunction in TMJ and the orofacial area.

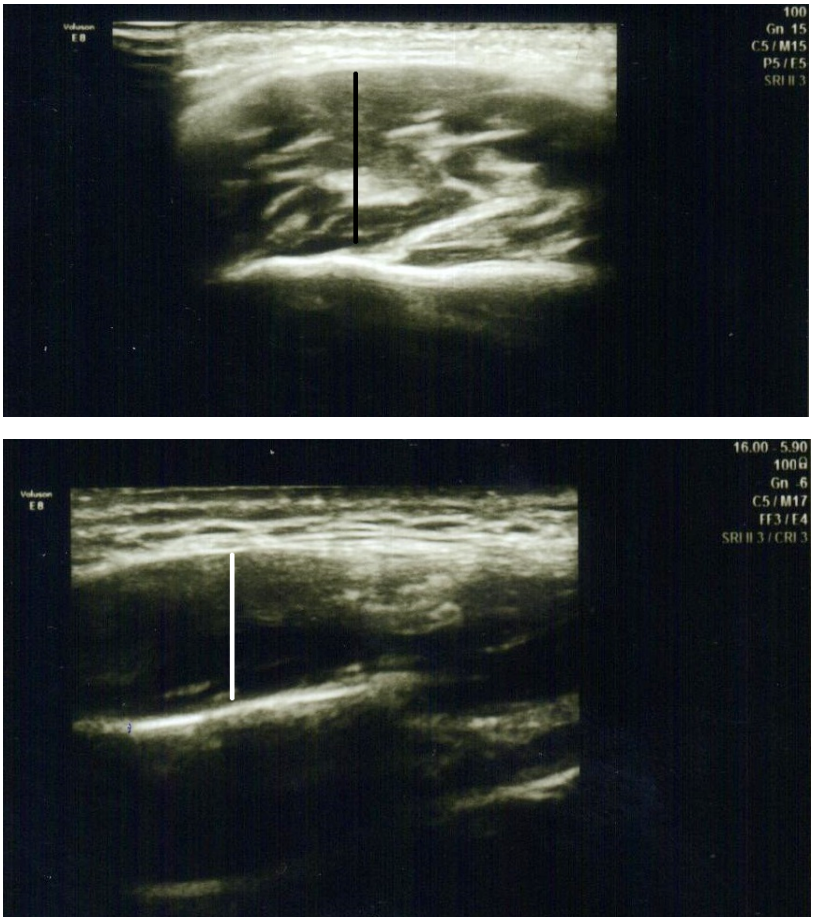

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Figure 1. Ultrasonography of masseter muscle

Right: Masseter muscle in rest position, Left: Masseter muscle in clenched position.

The exclusion criteria for both groups were having a history of surgery in the orofacial area during the last 6 months, pregnancy, systematic diseases, such as Parkinson, multiple sclerosis, arthritis rheumatoid, positional vertigo, and severe deformity in the jaw or face region.

Study instruments and procedures

In this study, all images were taken with subjects in a sitting position. The participants were asked to sit in an upright position on a chair with back support, look forward, and hold their heads in a neutral position. A diagnostic ultrasound apparatus (Voluson EB) with a 10 to 14 MHz, 26-mm linear transducer was used to acquire images in B–mode. The imaging of the masseter muscle was performed in 2 positions, including rest and contraction. The selection of intercuspal position was based on rest and contraction positions. The thickness of the middle part of the masseter muscle was measured. For imaging of the masseter muscle at rest, the subjects were asked to close their mouth without holding their teeth together.

To image the masseter muscle in rest position after the application of lubricating gel, the linear transducer was transversally positioned parallel to the mandibular body between the zygomatic arch and the superior border of the mandibular body [14]. To image the masseter muscle in a contraction position, the participants were instructed to perform clenching using holding the teeth together as maximally as possible without feeling pain. For the clenched intercuspal position, the imaging protocol was similar to the rest position. The thickness of the masseter muscles was measured as the distance between the outer and inner fascia of the muscle [14] (Figure 1).

Imaging of the LPM was performed in the mouth opening position [10, 14]. The participants were asked to open their mouths as maximally as possible without feeling pain. The linear transducer was transversally placed under the zygomatic arch, anterior to TMJ. The lateral wedge of the probe was then moved slightly in the superior-inferior direction until a clear picture of the LPM could

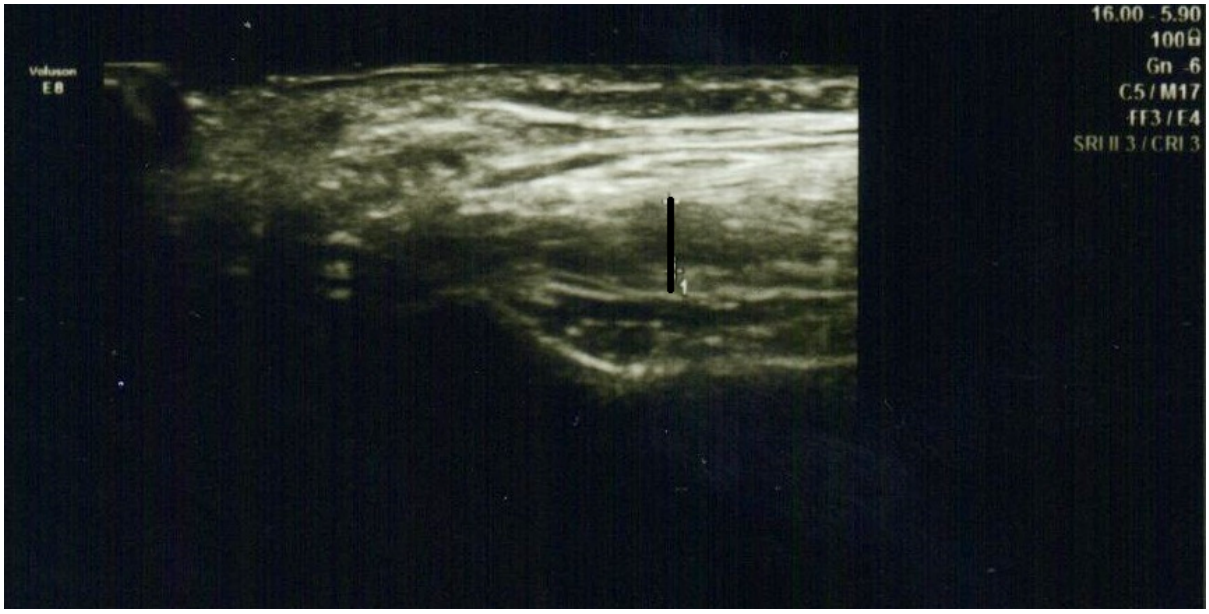

Figure 2. Ultrasonography of lateral pterygoid muscle

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be visualized. The thickness was determined as the distance between the superior and inferior fascia (Figure 2).

In our study, all pictures were recorded by an experienced radiologist specialist who has more than 20 years of experience in this field. Imaging for each of the muscles (masseter in rest and contraction positions, LPM in mouth opening position) was performed 3 times in each session, and the mean of 3 repetitions was used for the statistical analyses. To perform the measuring in the second session, the same protocol in the first session was used with a 2-day interval.

Images were saved and analyzed offline by a blinded investigator using Image-J software, version 1.52. The measurements in this intra-rater reliability study included the thickness of the masseter muscle at rest and contraction and the thickness of the LPM. The unit of measurement was mm.

Data analysis

Statistical analyses were conducted using the SPSS software, version 20. For the intra-rater reliability study, relative and absolute reliability variables were calculated. The relative reliability variable was $ICC_{(3, 3)}$, two-way mixed average measures (absolute agreement) with 95% confidence intervals (CI). ICC values were interpreted as follows: ICC ≤0.49=poor reliability; 0.5≤ ICC ≤0.74=moderate reliability; 0.75≤ ICC ≤0.89=good reliability; and ICC ≥0.90=excellent reliability [24].

Absolute reliability variables included the standard error of measurement (SEM) with 95% CI (SEM=SD×√[1-ICC]), minimal detectable change (MDC) with 95% CI (MDC=1.96×SEM×√2), and limits of agreement (LOA) with 95% CI [24]. Additionally, the Bland-Altman plots were used to visualize the limits of agreement.

Data distribution was checked using the Shapiro-Wilk test. All variables had a normal distribution. The independent sample t-test was used to compare the measurements between the two groups, and the Cohen d effect size was used to determine the size of between-group differences. The effect sizes were interpreted as follows: Effect size ≤0.19=trivial zone; 0.2≤ effect size ≤0.49=small zone; 0.5≤ effect size ≤0.79=medium zone; effect size ≥0.8=large zone [25]. A P<0.05 was considered significant.

3. Results

The demographic data of the two study groups are summarized in Table 1. A total of 86.7% of participants in each group were females and 20% of the TMD group and 6.7% of the asymptomatic group reported bruxism. Meanwhile, 20% of the symptomatic group had bilateral TMD. Additionally, the disability score of the TMD patients was 51.17% (20.47 out of 40). Based on the independent sample t-test and the chi-squared test, the groups were similar at baseline data and no significant differences were observed between the TMD group and the asymptomatic group regarding age, height, weight, body mass index, and sex.

Table 1. Descriptive data of the participants (n=15)

Variables		Mean±SD/No. (%)		P
		TMD	Asymptomatic	
Age (y)		33.13±4.5	32.07±3.24	0.44
Weight (kg)		64.73±4.56	65.93±4.95	0.49
Height (cm)		165.13±4.73	164.93±3.51	0.90
BMI (kg/m^2)		23.79±2.12	24.26±2	0.54
TMD index questionnaire (of 40)		20.47±3.25	-	-
TMD index questionnaire (%)		51.17(8.12)	-	-
Sex	Males Females	2(13.3) 13(86.7)	2(13.3) 13(86.7)	0.9
	Unilateral TMD Bilateral TMD	12(80) 3(20)	- -	-
Bruxism	Yes No	3(20) 12(80)	1(6.7) 14(93.3)	-

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Abbreviations: BMI: Body mass index; cm: Centimeter; kg: Kilogram; SD: Standard deviation; TMD: Temporomandibular disorder.

The results of intra-rater reliability for measurements of the masseter muscle and the LPM in both groups are presented in Table 2. The $ICC_{(3, 3)}$ values for all measurements demonstrated good to excellent intra-rater reliability (ICC≥0.75). For the masseter thickness in the rest position, the $ICC_{(3, 3)}$ values (95% CI) ranged from 0.66 to 0.96 and from 0.40 to 0.97, respectively, and for the masseter thickness in the contraction position,$ICC_{(3, 3)}$ values ranged from 0.42 to 0.93 and from 0.43 to 0.93 for the TMD group and the asymptomatic group, respectively. The $ICC_{(3, 3)}$ values for the LPM thickness ranged from 0.57 to 0.97 and from 0.55 to 0.95 for the TMD group and the asymptomatic group, respectively.

The MDC values of the TMD group were 0.50 mm, 0.53 mm, and 0.61 mm. In the asymptomatic group, the values were 0.66, 0.78, and 0.58 mm for mater muscle thickness in rest and contraction positions and LPM. Additionally, the Bland Altman plots presented the limit of agreements between the two sessions for all measurements (Figures 3 and 4). The LOA plots showed a small difference between the sessions. However, the LOA plots were wider for the asymptomatic group compared to the TMD group. In both groups, the greatest difference between the two sessions was related to the thickness of the masseter muscle in the contraction position.

Between-group comparisons

The results of the between-group comparisons are provided in Table 3. The independent sample t-test showed that, compared to the asymptomatic group, the thickness of the masseter muscle in the rest position was significantly greater in the TMD group. Additionally, the effect size confirmed a large difference between the two groups. While no significant differences were found between the groups for the thickness of the masseter muscle in contraction position and LPM. The effect size revealed a medium difference between the groups with a wide CI for LPM thickness.

4. Discussion

This study was designed to assess the reliability of sonography measurements of the masticatory muscles in TMD and asymptomatic subjects. Additionally, the groups were also compared. The results of this study suggested that ultrasonography measurements of masticatory muscles in TMD and asymptomatic subjects exhibit good to excellent reliability.

Intra-rater reliability

Extensive research has demonstrated that ultrasonography is a reliable tool for measuring muscle morphology. However, little attention has been paid to determining

Table 2. Intra-rater reliability results

Variables	Group	Mean±SD		$ICC_{(3,3)}$ (95%CI)	SEM (95%CI)	MDC (95%CI)	LOA (95%CI)
		Session 1	Session 2				
Masseter (rest) (mm)	TMD group	12.73±0.55	12.83±0.51	0.88 (0.66-0.96)	0.18 (0.11-0.31)	0.50 (0.3-0.86)	-0.10 (-0.78-0.57)
	Asymptomatic group	11.75±0.66	11.42±0.79	0.89 (0.40-0.97)	0.24 (0.12-0.56)	0.66 (0.33-1.55)	0.33 (-0.38-1.04)
Masseter (contraction) (mm)	TMD group	13.28±0.44	13.43±0.43	0.80 (0.42-0.93)	0.19 (0.11-0.33)	0.53 (0.3-0.91)	-0.15 (-0.83-0.52)
	Asymptomatic group	13.20±0.68	13.40±0.59	0.80 (0.43-0.93)	0.28 (0.17-0.48)	https://jmr.tums.ac.ir/ind https://jmr.tums.ac.ir/index.php/jmr/article/view/728 ex.php/jmr/article/view/728 0.78 (0.47-1.33)	-0.20 (-1.19-0.79)
LPM (mm)	TMD group	13.06±0.64	12.79±0.75	0.90 (0.57-0.97)	0.22 (0.12-0.45)	0.61 (0.33-1.25)	0.2 (-0.44-0.98)
	Asymptomatic group	12.74±0.58	12.53±0.57	0.86 (0.55-0.95)	0.21 (0.13-0.38)	0.58 (0.36-1.05)	0.27 (-0.44-0.98)

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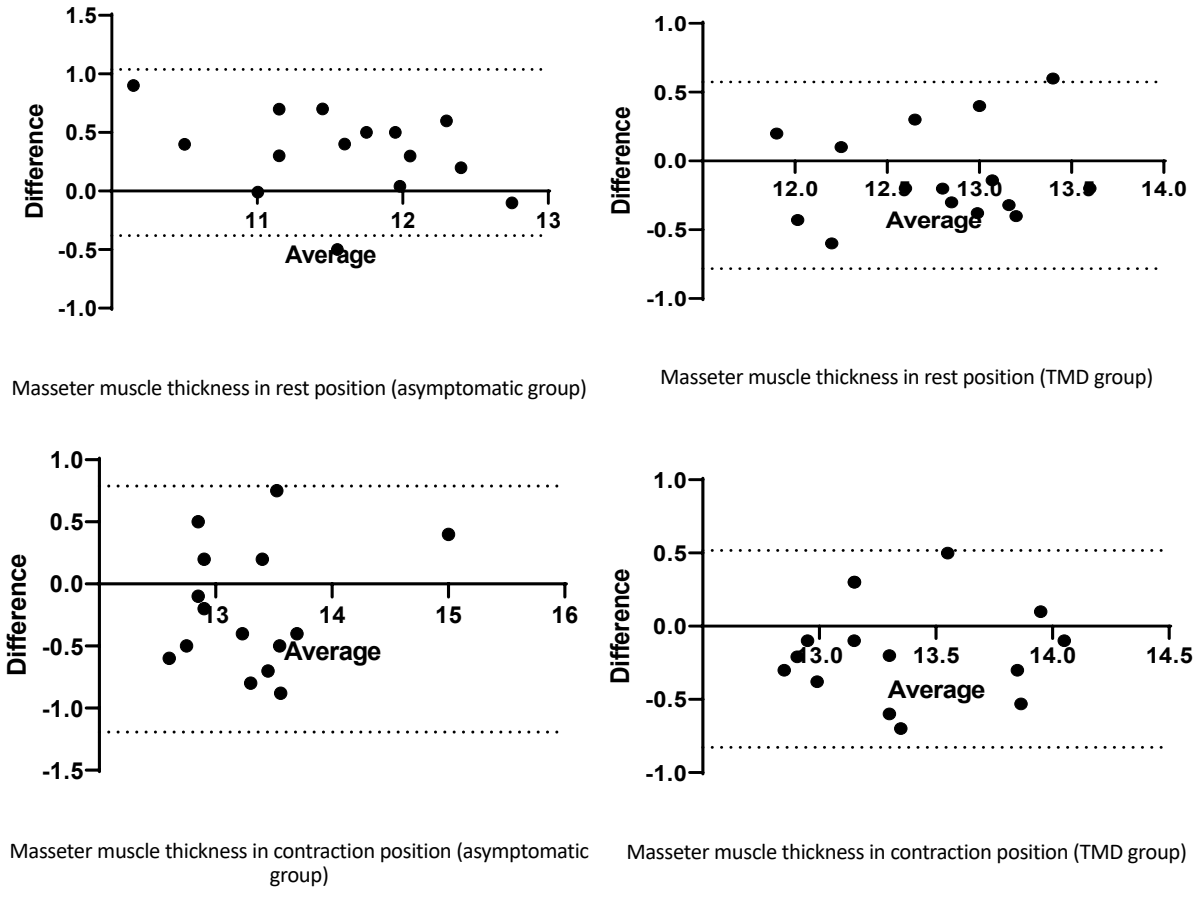
Abbreviations: CI, confidence interval; ICC, intraclass correlation coefficient; LPM, lateral pterygoid muscle; LOA, limit of agreement; MDC, minimal detectable change; mm, millimeter; SD, standard deviation; SEM, standard error of measurement; TMD, Temporomandibular disorder.



Figuue 3. Bland-altman plot showing the agreement between first session and second session for masseter muscle thickness in rest (asymptomatic group [upper left] and tmd group [upper right]) and contraction positions (asymptomatic group [lower left) and tmd group [lower right])

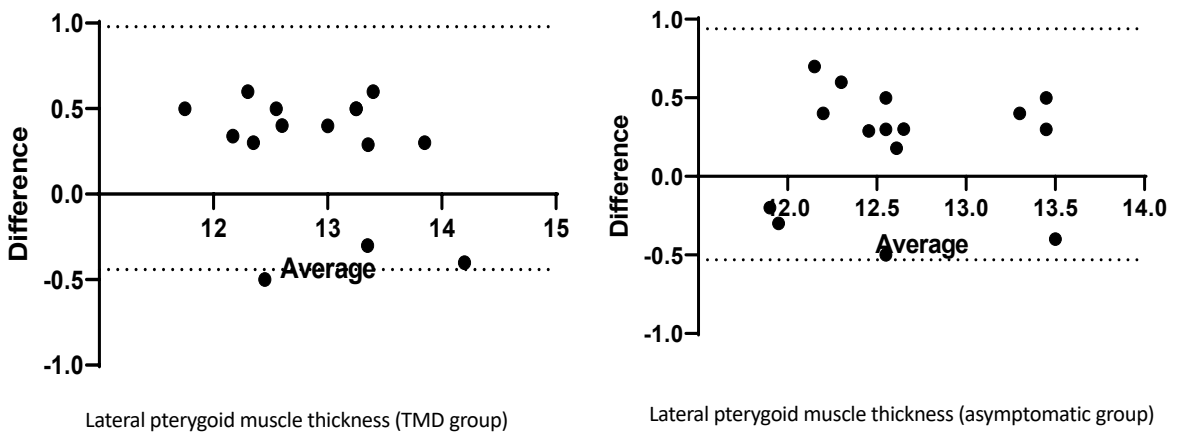


Figure 4. Bland-altman plot showing the agreement between first session and second session for lateral pterygoid muscle thickness

Table 3. Results of independent sample t-test and Cohen d effect size with 95% confidence interval

Variables	P	Mean Difference (95% CI) TMD Group-asymptomatic Group	Cohen d (95% CI)
Masseter (rest) (mm) (session 1)	0.0001	0.98 (0.52-1.44)	1.6 (0.76-0.2.42)
Masseter (rest) (mm) (session 2)	<0.001	1.41 (0.91-1.91)	2.11 (1.2-30)
Masseter (contraction) (mm) (session 1)	0.72	0.08 (-0.35-0.5)	0.13 (-0.58-0.85)
Masseter (contraction) (mm) (session 2)	0.88	0.03 (-0.36-0.42)	0.06 (-0.66-0.77)
LPM (mm) (session 1)	0.17	0.32 (-0.14-0.77)	0.51 (-0.22-1.24)
LPM (mm) (session 2)	0.25	0.29 (-0.22-0.8)	0.44 (-0.3-1.17)

Abbreviations: CI: Confidence interval; LPM: Lateral pterygoid muscle; mm: Millimeter.

the reliability of sonography measurements of masticatory muscles in subjects with and without TMD up to now [14, 20, 21, 26]. Chang et al. [14] investigated the intra-rater reliability of the thickness of superficial and deep masticatory muscle in asymptomatic participants. They measured the reliability of masseter thickness in 3 sections, including upper, middle, and lower along with 3 conditions, including rest, maximal mouth opening, and clenching position. The ICC values for the thickness of the upper part masseter were 0.86 and 0.88 in rest and clenching positions, respectively [14]. The ICC values of the middle part of the muscle in the rest and clenching position were 0.82 and 0.83, respectively. They also reported ICC values of 0.75 and 0.69 for the lower part of the muscle in the rest and clenching position, respectively [14]. In another study, Barotsis et al. [20] assessed the reliability of ultrasonography measurements of the masseter muscle in healthy subjects in transverse and longitudinal planes. They investigated the reliability of measurements in the rest position and the measurements were repeated after 1, 6, and 24 h. The ICC values ranged from 0.29 to 0.77 in the longitudinal section and the range of ICC values in the transverse section was from 0.36 to 0.75 [20].

In our study, only the thickness of the middle part of the masseter muscle was measured in conditions of rest and clenched position. The $ICC_{(3, 3)}$ values of the middle part of the masseter muscle in asymptomatic subjects were 0.89 and 0.80 in rest and clenched positions, respectively.

In addition, relative reliability values showed good reliability for masseter thickness in rest ($ICC_{(3, 3)}$=0.80) and clenched positions ($ICC_{(3, 3)}$=0.80) in TMD subjects. These results are consistent with the findings of Bertram et al. [21] who determined the reliability of masseter muscle thickness using sonography in TMD subjects. They measured the thickness of the masseter muscle in the upper, middle, and lower sections. The ICC values in the middle part of the muscle in relaxed and contracted positions were 0.88 and 0.92, respectively [21].

In our study, in addition to the relative parameter, absolute reliability parameters, including SEM, MDC, and LOA were also calculated. The SEM values for measurements of the masseter muscle in the asymptomatic subjects were 0.24 mm and 0.28 mm in rest and contraction positions, respectively. Compared to the asymptomatic subjects, the SEM values in TMD subjects were less (SEM in rest position=0.18 mm and clenched position=0.19 mm). Similar to the SEM values, the MDC values in the TMD group were lower compared to the asymptomatic group. In the present study, the threshold of MDC values for all variables in both groups was more than the differences between measurements in 2 sessions. This observation confirms that the measurements in the present study were reliable. The MDC values of the masseter muscle thickness in the TMD group at rest and contraction positions were 0.50 mm and 0.53 mm, respectively. The MDC values in the asymptomatic group at rest and contraction positions were 0.66 mm and 0.78 mm, respectively. Absolut reliability values in our study were lower than the study of Chang et al. [14]. In their study, SEM and MDC values of the middle part of the muscle in the rest position were 0.86 mm and 2.36 mm and in the contraction position were 0.89 mm and 2.47 mm, respectively [14]. The lower absolute reliability parameters indicate greater accuracy of measurements [27].

Additionally, LOA and the Bland-Altman plots were used to assess the reliability of masseter measurements. These items also confirmed the reliability measurements. LOA plots were wider for the asymptomatic group compared to the TMD group. Both groups had the greatest

difference between the 2 sessions for the thickness of the masseter muscle in the contraction position.

In addition to the masseter muscle, the reliability of sonography measurements of LPM was assessed. The imaging process of this muscle is difficult. Deep location, being triangular shape, and covering by mandibular ramus in closed mouth condition are possible explanations for this result [14]. Hence, compared to the masseter muscle, fewer studies have investigated the reliability of measurements of the LPM. In this study, imaging of the LPM was performed in maximal mouth opening, and in contrast to the study of Chang et al. [14], a linear transducer was used. In our study, all imaging was performed by an experienced radiologist who was familiar with the specific ultrasound imaging (USI) machine and protocol used in this study. An experienced examiner, using a USI machine with higher quality can be the possible reason for recording the pictures of the LPM with the linear probe.

The ICC value for the thickness of LPM in our study was greater than the results of Chang et al. [14]. They reported an ICC value of 0.63, ranging from 0.28 to 0.84 in the asymptomatic group, while in our study, the TMD group presented excellent (ICC=0.90) and the asymptomatic group presented good reliability (ICC=0.88) for measuring the thickness of the LPM. The SEM and MDC values in the TMD subjects ranged from 0.12 mm to 0.45 mm and from 0.33 mm to 1.25 mm, respectively and the range of SEM and MDC values in the asymptomatic group was from 0.13 mm to 0.38 mm, and 0.36 mm to 1.05 mm, respectively. The Bland-Altman plots also visualized a narrow range for LOA. Compared to the present study, Chang et al. [14] reported greater SEM (0.58 mm) and MDC (1.62 mm) values for the thickness of LPM in the asymptomatic group.

Between-group comparisons

In addition to assessing intra-rater reliability as the first objective, the second purpose of this study was to compare the masseter and LPM thickness between the subjects with and without TMD.

Some studies have investigated the thickness of the masseter and LPM muscles in subjects with and without TMD. The thickness of masticatory muscles was reported 14.85 mm and 15.63 mm for the middle part of the masseter in rest and clenched positions, respectively, and 14.0 mm for the LPM in asymptomatic subjects by Chang et al. [14]. In the study of Ariji et al. [28], the thickness of the masseter muscle on the symptomatic and asymptomatic sides was as 9.1 mm and 8.1 mm, respectively. In another study by Ariji et al. [11], the masseter muscle was reported 9.7 mm in TMD patients and 8.28 in the asymptomatic group. Odkhuu [17] et al. measured the masseter muscle thickness in males and females in the rest and clenched positions. The thickness of the masseter muscle was 11.18 mm and 10.5 mm in the rest position and 13.71 mm and 12.5 mm in the clenched position in males and females with TMD, respectively. Additionally, Eren et al. [10] measured masticatory muscle thickness in subjects with and without bruxism. They reported that the mean values of the thickness of the masseter in the bruxism group are 7.6 mm and 11.81 mm and in the control group are 7.9 mm and 12.89 mm in the rest and clenching positions, respectively [10]. Additionally, the thickness of the LPM was reported 5.6 mm in the bruxism group and 5.52 in the subjects without bruxism [10].

The above-mentioned studies have reported various values for the thickness of the masticatory muscles. These differences between the studies may partly be explained by the sonography measurements of masticatory muscles depending on the location of measurements, transducer position, and the level of pain during assessments [26]. Additionally, the demographic characteristics of participants are one of the factors that can affect the masticatory muscle measurements [14, 29, 30].

In the present study, the measurements of the masseter muscle were performed in the middle section of the muscle in 2 conditions, namely rest and contraction, and the imaging of LPM was performed in the mouth opening position. The mean values of thickness of masseter muscle in rest and contraction and LPM in the asymptomatic group in the first session were 11.5 mm, 13.20 mm, and 12.4 mm, respectively, and in the TMD group was 12.3 mm, 13.28 mm, and 13.06 mm, respectively. Meanwhile, in the second session, the values were 11.42 mm, 13.40 mm, and 12.53 mm in the asymptomatic group, and 12.83 mm, 13.43 mm, and 12.79 mm in the TMD group.

Between-group comparisons revealed that, compared to the asymptomatic group, the TMD group had thicker masseter muscle in the rest position. Also, the size of the difference based on the Cohen d was large [31]. Additionally, while the thickness of masseter muscle in contraction and LPM in TMD subjects was greater than in the asymptomatic group, these between-group differences were not statistically significant. Our findings are in line with previous studies that have reported the thickness of the masticatory muscles can be influenced by TMD [8-12, 15, 16]. The possible mechanisms for

these findings include continuous contraction, overuse activity, compensatory pattern, and edematous change of the muscles following TMD and functional habits, such as bruxism [10, 11, 14, 32, 33]. Additionally, in our study, the TMD subjects had thicker muscles in the contraction position compared to the asymptomatic subjects, although the differences were not significant. There are some explanations for this finding. In addition to the sample size that can be effective on statistical significance, based on previous studies, continuous contraction in TMD patients can increase the muscle thickness in rest position compared to the asymptomatic subjects. However, in the contraction position, pain and inflammation may decrease the ability of voluntary contraction in TMD patients; therefore, the difference in the contracted position was not significant.

5. Conclusion

The results of the intra-reliability study showed that ultrasonography is a reliable method for measuring the thickness of the masticatory muscles and can be used for therapy and research objectives in subjects with TMD. Additionally, between-group comparisons showed that the thickness of the masticatory muscles varied between the TMD subjects and asymptomatic subjects.

Study limitations

There were some limitations to our study. One limitation of the study is that there are different types of TMD, and we included patients with TMD; accordingly, the results cannot be attributed to a special type of TMDs.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Iran University of Medical Sciences (Code: IR.IUMS.REC.1397.1054). All participants signed the informed consent.

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Authors' contributions

Conceptualization, methodology and final approval: All authors; Initial draft: Leila Alizadeh and Khaled Rezaie.

Conflict of interest

The authors declared no conflict of interest.

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